# INFLUENCE DE LA VARIABILITÉ ENVIRONNEMENTALE SUR LES TRAITS MATERNELS D'UN GRAND MAMMIFÈRE, LE MOUFLON D'AMÉRIQUE

par

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À ma famille, qui a toujours cru en moi

# SOMMAIRE

Déterminer comment les traits maternels s'ajustent aux variations de l'environnement est important puisque les conditions en début de vie sont cruciales pour la survie et la reproduction futures d'un organisme. Chez les mammifères, particulièrement les espèces longévives, la période de soins maternels est souvent caractérisée par la lactation, qui débute au moment de la naissance et prend fin pendant le processus du sevrage. La lactation peut durer plusieurs mois, comme elle peut représenter plusieurs années, par exemple chez certains primates et les humains. Parmi les causes connues de variation des traits liés à la reproduction sont souvent cités les changements phénologiques. Ces changements dans les cycles d'évènements biologiques entraînent souvent un décalage dans les fenêtres temporelles qui servent de repères pour synchroniser la reproduction aux conditions environnementales optimales. Par contre, encore à ce jour, peu d'études ont réellement testé la supposition selon laquelle les naissances coïncident avec la disponibilité maximale de nourriture au printemps chez les mammifères. L'objectif principal de cette thèse est donc de clarifier les liens entre les traits liés à la reproduction et la variabilité environnementale, en analysant principalement les dates de mise-bas, la survie néonatale et la composition du lait. Pour ce faire, la population de mouflon d'Amérique (*Ovis canadensis*) de Ram Mountain, Alberta, Canada, est utilisée comme modèle d'étude. Cette population est suivie individuellement depuis plus de 40 ans et les traits d'histoire de vie sont connus pour la grande majorité des brebis et leur agneau.

Dans un contexte de changements climatiques, l'environnement peut être variable et imprévisible, et les organismes peuvent avoir à ajuster leur cycle de reproduction pour qu'il soit toujours optimal. Une façon de s'ajuster est par la plasticité phénotypique (l'autre étant par la microévolution ou encore la migration) ; elle est définie par l'expression de plusieurs phénotypes par un seul génotype, ou individu. À Ram Mountain, les dates de naissance des agneaux ont été devancées d'une dizaine de jours en 26 ans. Nous avons posé l'hypothèse que cette tendance populationnelle s'explique par plasticité phénotypique individuelle en réponse aux changements environnementaux qui ont lieu dans l'aire d'étude (Chapitre 2). La durée de la gestation étant peu flexible chez le mouflon, nous avons présumé que les dates de naissance découlaient directement des dates de conception qui ont lieu à l'automne. Mes résultats suggèrent que les brebis décalent les dates de naissance avec l'augmentation des précipitations

quotidiennes par plasticité phénotypique. Les dates de naissance sont devancées avec l'augmentation des températures au niveau populationnel, mais aucun effet de la densité ou du climat (le PDO) n'est détecté. La capacité des brebis à s'adapter aux changements rapides de leur environnement s'expliquerait en partie par plasticité phénotypique.

Le moment de la naissance détermine le début de la lactation, une adaptation unique aux mammifères qui permet à la mère de faire un transfert d'énergie direct important à la survie de son jeune. Bien que la composition du lait ait été largement décrite chez une diversité d'espèces de mammifères, très peu d'études s'intéressent aux différences individuelles en composition du lait et à leurs conséquences en valeur adaptative. Ceci peut être dû en partie à la complexité de ce système physiologique, qui nécessite plusieurs marqueurs pour être bien décrit. De plus, très peu de systèmes d'étude en milieu sauvage offrent la possibilité de récolter de tels échantillons sur plusieurs individus marqués. Le chapitre 3 aborde une problématique que beaucoup d'écologistes, incluant les écophysiologistes, rencontrent dans le traitement de leurs données : une grande quantité de traits mesurés sur un faible nombre d'individus. Par une approche méthodologique, je propose de décomposer les différentes échelles biologiques qui influencent les marqueurs physiologiques, et qui expliquent en partie leur complexité. J'utilise cette approche pour tester l'hypothèse selon laquelle la composition du lait a des conséquences sur le succès reproducteur à court terme. En utilisant des mesures répétées de femelles marquées sur 6 ans, j'ai voulu 1) évaluer la présence de différences répétables en composition du lait entre les brebis, 2) déterminer l'importance relative des différences interannuelles et interindividuelles en composition du lait, et 3) lier la physiologie aux traits d'histoire de vie importants pour la valeur adaptative et 4), vérifier si la composition du lait est corrélée aux changements en masse des femelles et à la survie au sevrage des agneaux. Pour répondre à ces questions, nous avons adapté une forme de modèles multivariées à la nature des données de composition du lait. Cette approche permet de simplifier grandement la décomposition de la matrice de (co)variances en utilisant un nombre réduit de variables latentes. Mes résultats suggèrent que les variations interannuelles expliquent la majorité de la variation en composition du lait. Lorsque les marqueurs physiologiques du lait sont mis en lien avec les traits maternels comme la masse, ou encore, le changement en masse, qui sont déterminants pour la valeur adaptative chez le mouflon, une seule composante du lait montre une corrélation avec ces traits : la concentration en acides gras. Toutefois, la source de variation de cette composante, ainsi que les protéines, sucres et minéraux, reste en grande partie environnementale. Aucun effet de la composition du lait sur la survie au sevrage n'a été détecté, ce qui confirme la difficulté à mettre en lien la complexité

physiologique et les traits liés à la valeur adaptative.

Enfin, je me suis intéressée à l'influence de la phénologie de la végétation sur le développement et la survie en bas âge des agneaux (Chapitre 4). Avec une augmentation des températures globales, les dates de ponte, d'émergence de l'hibernation et de mise-bas surviennent de plus en plus tôt chez une majorité d'espèces animales. De plus, l'émergence hâtive de la végétation peut entraîner de plus longues périodes de disponibilité de nourriture pour les organismes qui en dépendent. En conséquence, on observe des changements phénotypiques liés à ces changements. Dans ce chapitre, je teste l'hypothèse selon laquelle les naissances et le début de la lactation - activité coûteuse pour les femelles - sont synchronisés avec la période où la nourriture est abondante pour permettre aux mères de soutenir ce coûteux transfert d'énergie. Je quantifie les causes et les conséquences biologiques de la variabilité interannuelle en survie néonatale, composition du lait, masse au sevrage et survie au premier hiver. Un suivi régulier des brebis marquées permet la détermination de leur statut de lactation et donc, de leur succès reproducteur. À l'aide de données de végétation extraites d'images satellitaires, j'ai montré que de longues périodes de disponibilité de la végétation améliorent la survie néonatale et augmentent la concentration en gras et minéraux du lait. Les agneaux nés tard relativement à la date d'émergence des plantes souffrent davantage de mortalité néonatale que les agneaux nés tôt, suggérant un impact potentiel d'un décalage avec la croissance végétative. Une forte prédation en été diminue la survie néonatale au printemps suivant. Finalement, bien que la composition du lait change avec la phénologie de la végétation, je ne détecte qu'un effet marginal de la composition du lait sur la masse au sevrage et aucun effet, direct ou indirect, sur la survie à un an des agneaux.

En résumé, les résultats de ma thèse suggèrent que la variabilité environnementale influence l'histoire de vie du mouflon à différents stades de son développement. Les changements phénologiques peuvent donc avoir des impacts au niveau du recrutement en influençant la qualité des soins maternels fournis à la progéniture. Mes recherches permettent de mieux comprendre le potentiel d'ajustement des espèces face aux changements d'origines anthropiques, dont les plus médiatisés sont certainement les changements climatiques. Mes travaux soulignent aussi l'importance des suivis individuels de populations d'animaux sauvages, sans lesquels les études en écologie évolutive seraient impossibles. Finalement, mes recherchent montrent le potentiel des approches intégratives et mécanistiques pour tester des hypothèses écologiques basées sur des données complexes.

**Mots clés :** effets maternels, grands vertébrés, lactation, phénologie, plasticité phénotypique

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# LISTE DES ABRÉVIATIONS

**GPP :** Productivité primaire (*Gross Primary Productivity*)

**MHM :** Un modèle hiérarchique multivarié, en référence aux modèles conjoints utilisés en écologie des communautés

**NAO :** Oscillation nord-atlantique (*North Atlantic Oscillation*)

**PDO :** Oscillation décennale du Pacifique (*Pacific Decadal Oscillation*)

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CHAPITRE 1

# INTRODUCTION GÉNÉRALE

## 1.1 L'Homme et l'Anthropocène

À l'ère de l'Anthropocène, la biodiversité fait face à cinq grandes forces de bouleversement : la surexploitation, les modifications d'habitats, les changements climatiques, les espèces invasives et la pollution (Steffen et al., 2011 ; Pelletier et Coltman, 2018 ; Díaz et al., 2019). La force de ces changements est telle qu'elle mène à des changements phénotypiques, à des adaptations locales et des changements génotypiques rapides mais parfois à des extinctions. En réponse aux activités induites par l'Homme, la microévolution contemporaine a bien lieu et l'Homme serait une des plus grandes forces évolutives sur terre, façonnant les traits de plusieurs espèces animales et végétales (Palumbi, 2001).

Parmi les facteurs influençant la biodiversité, les changements climatiques surviennent à une vitesse inégalée, tant en milieu marin que terrestre (Thackeray et al., 2010). Les conséquences des changements climatiques sont multiples, et incluent l'augmentation des températures moyennes, des changements importants en précipitations (Siepielski et al., 2017), des chutes de neige tardives dans les milieux tempérés et une augmentation de la fréquence d'évènements extrêmes ayant des conséquences écologiques importantes (Marrot et al., 2017 ; Ummenhofer et Meehl, 2017). Les températures globales auraient augmenté de 0,87°C entre la période pré-industrielle et les années 2006-2015 (Allen et al., 2018). Des changements dans la distribution des espèces (Parmesan et al., 1999 ; Thomas et Lennon, 1999 ; Parmesan et Yohe, 2003 ; Parmesan, 2006) et dans la distribution des traits phénotypiques (Marrot et al., 2017) sont observés, allant jusqu'à augmenter la force de sélection sur des traits liés à la valeur adaptative (e.g., la date de ponte ; Reed et al. (2013) ; Marrot et al. (2017)). En conséquence, la dynamique de toute une population sauvage peut être affectée (Jenouvrier et al., 2009), particulièrement chez les espèces habitant des écosystèmes « extrêmes » situés à haute altitude ou latitude (Parmesan, 2006 ; Ozgul et al., 2010).

Les traits phénologiques, qui suivent une séquence d'évènements saisonniers et une fenêtre temporelle précise (voir encadré 1), sont particulièrement sensibles aux changements environnementaux (Parmesan, 2006). Les changements climatiques posent aux organismes le défi de synchroniser leurs activités vitales avec la disponibilité changeante des ressources saisonnières (Parmesan, 2006). Initialement développée par un biologiste des pêcheries, David Cushing, l'hypothèse du « match/mismatch » visait à expliquer la croissance et la survie des larves de morue (*Gadus morhua*) en fonction de leur synchronie avec leur nourriture, le plancton (Gushing et Dickson, 1977). Cette hypothèse a rapidement été étendue au milieu terrestre pour expliquer comment les cycles climatiques, par exemple l'Oscillation Nord-Atlantique, déterminent la synchronie entre différents niveaux trophiques (Stenseth et Mysterud, 2002). Même si les notions de décalage et synchronie ne sont pas systématiquement testées de la même façon à travers le temps et les espèces, l'hypothèse a contribué au cadre conceptuel unifiant les études qui s'intéressent aux effets de changements phénologiques chez une diversité d'espèces animales et végétales.

Des 4000 espèces étudiées pour leur phénologie, 42% sont des plantes et près de la moitié (162) des 352 analyses de séries temporelles au niveau populationnel sont sur les oiseaux (Ummenhofer et Meehl, 2017). En combinant les jeux de données de (Parmesan et Yohe, 2003) et (Root et al., 2003), Parmesan (2007) rapportent que la hausse des températures globales depuis les années 1950 a contribué au devancement de traits phénologiques d'environ 2,8 jours par décennie dans l'hémisphère Nord (étendue de 1,1 à 9,6 jours par décennie), chez 203 espèces différentes. Les printemps hâtifs ont causé des modifications phénologiques dans la plupart des taxons y compris les arbres, qui ont devancé de 3,3 jours par décennie leur date de bourgeonnement (Parmesan, 2007). Les amphibiens seraient les plus sensibles au réchauffement, en devançant leur phénologie trois fois plus rapidement que les papillons et les oiseaux (7,6 vs 3,7 jours par décennie, respectivement) (Parmesan, 2007). Quantifier l'ampleur et la direction des réponses phénologiques aux changements climatiques est important pour comprendre si les réponses sont similaires entre niveaux trophiques et donc, synchronisées (Both et al., 2009). À ce jour, on ne comprend pas totalement pourquoi certaines espèces répondent aux changements climatiques en devançant leur phénologie, tandis que d'autres ne répondent pas ou encore, retardent leur phénologie en présence de réchauffement (Parmesan et Yohe, 2003 ; Root et al., 2003).

Encadré 1. Définir la phénologie

La phénologie est l'étude des séquences d'évènements saisonniers, aussi bien chez les organismes végétaux qu'animaux. Dans cette thèse, la phénologie fait référence aux stades de développement d'un organisme ou d'une population, au moment où ce stade coïncide avec un aspect particulier de l'environnement. Ces stades de développement sont souvent des évènements périodiques clés dans l'histoire de vie des organismes (Both et al., 2009 ; Lane et al., 2012), par exemple la floraison, la reproduction, les naissances, etc. Les naissances qui coïncident avec un changement important dans la disponibilité de végétation est l'exemple de phénologie qu'on retrouvera le plus souvent dans la thèse. Dans le contexte de cette thèse, particulièrement dans le Chapitre 4, le terme **phénologie du printemps** est souvent utilisé et fait référence aux dates de verdissement et de fonte des neiges, ainsi qu'à la longueur des saisons pendant lesquelles la végétation est abondante. Ces paramètres sont extraits de séries temporelles d'imagerie satellitaire.

Le terme **phénologie de la végétation** est aussi utilisé pour faire spécifiquement référence au cycle de vie annuel des plantes. Le terme **phénologie de la reproduction**, quant à lui, fait référence aux évènements clés du cycle de vie des animaux, notamment les naissances et la période de lactation. Lorsque la phénologie des plantes est abordée, elle l'est d'un point de vue de la communauté ou alors d'un trait (e.g. la date moyenne de bourgeonnement ou la date de productivité primaire maximale). Lorsque la phénologie des animaux est abordée, elle l'est davantage du point de vue d'un trait individuel (e.g. la date de mise-bas d'une femelle).

## 1.2 La phénologie de la reproduction

### 1.2.1 Les stratégies de reproduction

La théorie de l'histoire de vie prédit que lorsque les ressources sont limitées, les coûts énergétiques élevés d'activités vitales, telle la reproduction, peuvent entraîner des compromis entre les traits (van Noordwijk et de Jong, 1986 ; Stearns, 1992). Pour minimiser ces coûts, les organismes peuvent utiliser un continuum de stratégies allant de la reproduction sur « capital » (en

anglais *capital breeding*), qui dépend principalement des réserves corporelles, à la stratégie par allocation « journalière » (*income breeding*), qui dépend de l'ingestion de nourriture (Jönsson, 1997 ; Stephens et al., 2009). Ces stratégies de reproduction se basent sur la façon dont les individus acquièrent, entreposent et utilisent leurs ressources énergétiques, pour « financer » la reproduction. Il existe toutefois des espèces aux extrêmes du continuum, par exemple les phocidés. La plupart des phoques femelles sont des reproductrices sur « capital » ; la mère jeûne pendant la lactation et le lait est entièrement produit à partir de réserves de graisse (Oftedal et al., 1987). Les passereaux peuvent représenter l'autre « extrême » du continuum ; l'énergie requise pour la ponte et l'incubation dépend à 90% de l'énergie fournie par ingestion de nourriture pendant ces phases de la reproduction (Meijer et Drent, 1999). La plupart des espèces utilisent des stratégies intermédiaires entre les stratégies « journalière » et capitalistes (Meijer et Drent, 1999 ; Fletcher et al., 2013 ; Williams et al., 2017).

### 1.2.2 Changements en traits phénotypiques

Dans un contexte de changements climatiques, les stratégies de reproduction déterminent à quel point les traits phénologiques sont sensibles aux variations de l'environnement. Ainsi, chez les espèces à stratégie « journalière », tels plusieurs passereaux (Meijer et Drent, 1999), il est impératif que la ponte coïncide avec une période de nourriture abondante. À ceci s'ajoute l'avantage de pondre tôt dans la saison pour maximiser la taille de la couvée, la croissance et la survie des oisillons, ainsi que la valeur adaptative des parents (Verhulst et Nilsson, 2008). Or, la fenêtre temporelle utilisée par les adultes pour synchroniser la ponte et l'incubation des oeufs dépend largement de la photopériode et de la température au printemps (Bourret et al., 2015). En modifiant les séquences d'évènements saisonniers, en particulier le début du printemps dans les environnements tempérés et boréaux, les changements climatiques modifient les fenêtres temporelles avec lesquelles les organismes doivent synchroniser leur reproduction (Visser et Both, 2005 ; Parmesan, 2006 ; Thackeray et al., 2010). Certaines espèces d'oiseaux perdent donc leurs « repères » temporels, surtout si ceux-ci varient avec le climat (ce qui n'est pas le cas de la photopériode).

Dès la fin des années 1990, la littérature montrait déjà que certaines espèces d'oiseaux pondaient plus tôt (Crick et al., 1997) ou étendaient leur distribution vers le nord (Thomas et Lennon, 1999) en réponse aux augmentations de température. Cette littérature montrait des

impacts physiologiques chez les populations les moins bien synchronisées avec les ressources de nourriture, par exemple une augmentation de leur taux métabolique (Thomas et al., 2001). Chez ces espèces où la période allouée à la reproduction est courte, de légers décalages temporels dans la reproduction compromettent la condition des juvéniles et la survie des adultes (Thomas et al., 2001 ; Both et al., 2009). Chez la mésange bleue (*Cyanistes caeruleus*), le fait d'être synchronisé ou non avec le pic de disponibilité des chenilles a des conséquences directes sur la longévité des individus. Dans une population capable de se synchroniser avec l'éclosion des chenilles, la proportion d'adultes qui se reproduisent une deuxième année consécutive est de 52% ; chez une population « décalée » du pic de nourriture, cette proportion diminue à 25% (Thomas et al., 2001). En résumé, ces espèces à stratégie « journalière » doivent non-seulement synchroniser leur reproduction avec le pic d'abondance de nourriture, mais elles devront aussi réajuster leur « horloge biologique » pour minimiser le décalage entre la disponibilité de nourriture et la reproduction, décalage qui est exacerbé par les changements climatiques (Thomas et al., 2001).

En contraste, les espèces à stratégie par « capital » seraient moins sensibles aux variations de l'environnement, les ressources allouées à la reproduction étant majoritairement endogènes (Jönsson, 1997 ; Kerby et Post, 2013). Dans les milieux saisonniers, où la disponibilité des ressources est courte et les conditions environnementales souvent imprévisibles, la stratégie « capitaliste » permet de tamponner l'effet des variations saisonnières (Jönsson, 1997). De même, cette stratégie sous-entend qu'une partie seulement de l'énergie nécessaire à la reproduction sera puisée dans les réserves corporelles ; cette portion d'énergie peut être nécessaire à un moment crucial de la reproduction, par exemple en tout début de lactation si l'apport de nourriture est insuffisant pour pallier cette dépense énergétique. Ainsi, les ongulés les plus « capitalistes » dépendront davantage d'un apport journalier de nourriture pendant la lactation (Andersen et al., 2000), car cette dernière est très coûteuse d'un point de vue énergétique (Oftedal, 1985 ; Clutton-Brock et al., 1989). Positionner un organisme sur un gradient allant de « capitaliste » à « journalier » dépend donc du moment précis où les coûts associés à la reproduction sont mesurés (Stephens et al., 2009). Toutefois, les évidences montrant un ajustement des traits phénologiques chez les espèces « capitalistes » sont rares dans la littérature (Inouye et al., 2000 ; Ozgul et al., 2010 ; Boutin et Lane, 2014), même s'il est montré qu'un décalage dans la phénologie de différents niveaux trophiques aurait certainement des conséquences néfastes (Both et al., 2009) notamment des déclins de populations (Kerby et Post, 2013).

### 1.2.3 Conséquences démographiques des changements phénologiques

Chez les mammifères, la lactation occupe un rôle important dans la reproduction. Les traits liés à la lactation, par exemple la quantité ou la composition du lait, pourraient donc être influencés par des décalage de la phénologie. En réponse à l'augmentation des températures printanières, les marmottes à ventre jaune (*Marmota flaviventris*) émergent plus tôt d'hibernation (Ozgul et al., 2010). Ce devancement de la saison estivale permet aux marmottons de profiter d'une plus longue saison de croissance et de gagner davantage en masse, avant d'entrer en hibernation. Indirectement, le prolongement de la saison de croissance modifie possiblement la qualité du lait que les mères donnent à leurs jeunes, ce qui contribue potentiellement aussi à leur gain de masse. En conséquence, ces changements phénotypiques améliorent le succès reproducteur des adultes et diminuent la mortalité à plusieurs classes d'âge, si bien que la taille de population a augmenté dans les années les plus récentes de l'étude (Ozgul et al., 2010). Cette étude est une des rares à avoir démontré que les changements environnementaux, particulièrement un décalage de la phénologie, peuvent causer des changements phénotypiques et démographiques simultanés, ici au bénéfice de la croissance de la population.

Chez le chevreuil (*Capreolus capreolus*), des conséquences négatives d'un décalage de phénologie ont été mis en évidence (Plard et al., 2014). Chez cette espèce, la date de mise-bas est peu flexible (Plard et al., 2012). Les chevreuils mettent bas environ 1 mois avant la floraison, afin de profiter des jeunes pousses disponibles avant l'apparition des fleurs. Avec l'augmentation des températures printanières de 0,07 °C par année, les dates de floraison dans cette étude indiquent que la phénologie de la végétation s'est devancée de 0,6 jour par année entre 1985 et 2011 (Plard et al., 2014). Or, les dates de mise-bas n'ont pas suivi la tendance et le décalage entre la floraison et la mise-bas augmente donc de 0,54 jour par année. En conséquence, la survie par cohorte a diminué de 40% avec un décalage d'un mois et le taux de croissance de la population a diminué de 6% pendant l'étude (Plard et al., 2014). En résumé, que les changements globaux affectent les températures, les saisons de croissance de la végétation, ou encore, la fréquence de tempêtes printanières (Lane et al., 2012), les organismes subissent des conséquences écologiques de ces changements aux niveaux individuel et populationnel, avec des impacts potentiels sur la dynamique de population.

### 1.2.4 L'intégration des changements phénologiques

Comprendre comment les organismes intègrent les changements phénologiques est important pour prédire comment ils, particulièrement les endothermes, répondront aux changements climatiques. Toutefois, les mécanismes physiologiques de réponse sont, malheureusement, encore obscurs. Alors que la photopériode (rapport entre la longueur du jour et de la nuit) influence la saisonnalité, ou le « calendrier » des activités vitales sur l'échelle d'une année, elle ne peut expliquer les variations interannuelles dans ces mêmes évènements. La température est donc la variable la plus souvent corrélée aux variations interannuelles en saisonnalité (Caro et al., 2013). Pourtant, une réponse biologique à la variation de température n'implique pas nécessairement une causalité directe. Par exemple, les marmottes qui émergent de l'hibernation plus tôt dans les années plus chaudes répondent peut-être davantage à une floraison plus hâtive qu'à la température elle-même (Ozgul et al., 2010). Dans ce cas, comprendre comment la marmotte perçoit la température a peu d'intérêt car cette variable n'est pas *essentielle* à sa réponse biologique ; la date de floraison pourra directement être mesurée.

Pour d'autres espèces, comme la mésange charbonnière (*Parus major*), la température aura un effect causal sur la phénologie de reproduction (Visser et al., 2009). Caro et al. (2013) proposent quatre mécanismes par lesquels les variations de température sont perçus par le cerveau pour générer une réponse adaptée à la saisonnalité. Ces voies sont : 1) par les hormones thyroïdiennes, qui modulent la taille des organes reproducteurs (Wingfield et Sapolsky, 2003) ; 2) par la prolactine, importante pour la croissance des gonades, la ponte, la lactation et la mue ; 3) par la mélatonine, ou hormone du « sommeil », qui coordonne la saisonnalité chez les mammifères en agissant comme un miroir » interne qui reflète la longueur du jour ; et 4) par les neurones « thermosensibles » de la zone préoptique du cerveau, qui côtoient ceux du système neuronal responsable de la reproduction chez les vertébrés (Sharp, 2005 ; Caro et al., 2013). Les oiseaux possèdent des récepteurs photosensibles directement dans l'hypothalamus (Sharp, 2005), et ne nécessitent pas de mélatonine pour moduler les hormones thyroïdiennes. Bref, l'étude des différents mécanismes expliquant la saisonnalité chez les vertébrés reste un domaine où les connaissances ne cessent d'augmenter (Simonneaux, 2018).

## 1.3 Les mécanismes de réponses aux changements phénologiques

### 1.3.1 La dispersion et la migration

Chaque année, des millions d'animaux entreprennent des migrations sur plusieurs centaines, voire des milliers de kilomètres à travers le globe. Ces migrations sont un mécanisme pour pallier les variations de température et de phénologies qui surviennent tant en milieu marin que terrestre (Burrows et al., 2011). Certaines espèces seraient capables de « prévoir » les conditions à leur arrivée à leur aire estivale et ce, malgré une migration de plusieurs milliers de kilomètres ; en réalité, ces espèces traquent les variations dans la phénologie de la végétation à la base de leur alimentation (Merkle et al., 2016). Migrer, se disperser, coloniser et étendre sa distribution permettent de s'ajuster aux changements globaux ; les changements de distribution observés en milieu terrestre atteignent 6,1 km par décennie vers les pôles et de 1,4 à 28 km par décennie en milieu marin (Parmesan et Yohe, 2003). Au cours des 40 dernières années, les changements de distribution ont varié de 200 km (e.g., papillons, Parmesan et al. (1999)) à plus de 1000 km (e.g., copépodes, Beaugrand (2002)). Les migrations se font aussi en altitude, où la montée de la ligne des arbres a été notée (Walther et al., 2002). Certaines espèces ont pu coloniser des régions auparavant « froides », comme les anémones et les copépodes, alors que d'autres on rétréci leur distribution (Parmesan et Yohe, 2003), causant des changements importants au niveau des communautés. En somme, les changements observés dans la limite de distribution d'oiseaux (Thomas et Lennon, 1999), de papillons Parmesan et al. (1999) et de plantes (i.e., alpines, Parolo et Rossi (2008)) sont dans la direction prédite par les changements climatiques (Parmesan, 2006). Toutefois, tous les organismes ne possèdent pas des moyens de dispersion ou de déplacement efficaces et doivent utiliser d'autres mécanismes pour ajuster leur phénologie aux variations de leur environnement.

### 1.3.2 La plasticité phénotypique

Les organismes peuvent s'adapter aux variations de l'environnement par le biais de la plasticité phénotypique (Nussey et al., 2007 ; Charmantier et al., 2008) ou de la microévolution (Boutin et Lane, 2014 ; Merilä et Hendry, 2014), que nous aborderons plus loin. La plasticité phénotypique est l'expression de plusieurs phénotypes par un seul génotype, ou individu, en réponse à

différentes conditions environnementales (DeWitt et Scheiner, 2004). La plasticité permet un ajustement rapide du cycle vital et des traits morphologiques aux changements environnementaux (Gienapp et al., 2008 ; Boutin et Lane, 2014), lorsque ces traits sont flexibles. Par exemple, la plasticité phénotypique permet aux mésanges charbonnières d'ajuster la date de ponte en réponse à la hausse de la température du printemps (Charmantier et Gienapp, 2014). La plasticité phénotypique est généralement considérée comme le principal mécanisme permettant de faire face aux changements à court terme dans l'environnement, en particulier chez les oiseaux (voir Gienapp et al. (2008)).

Tous les individus d'une même population ne répondent pas de la même façon aux changements de leur environnement. L'approche des normes de réactions (Nussey et al., 2007) permet de distinguer les réponses populationnelles des réponses individuelles. Une norme de réaction est définie comme la trajectoire phénotypique d'un individu (ou de son génotype) selon un gradient environnemental (DeWitt et Scheiner, 2004). La pente de la norme de réaction illustre le degré de plasticité (Nussey et al., 2007), et lorsque la valeur de l'environnement est centré autour de sa moyenne, l'intercepte d'une norme de réaction exprime la réponse d'un individu dans un environnement moyen. Ainsi, dans l'étude de Charmantier et al. (2008), les mésanges ne varient pas individuellement dans le degré de plasticité ; elles expriment, en moyenne, toutes la même réponse plastique et les normes de réaction sont donc parallèles (Figure 1.1b). Dans une autre population de mésange, toutefois, il existe une variation considérable de la pente de la norme de réaction de la date de ponte en fonction de la température (Nussey, 2005). Les normes de réaction individuelles se différencient donc de la réaction moyenne de la population (Nussey, 2005), et on parlera de variation en plasticité ou de $I \times E$, pour *individual-by-environment interaction* (Figure 1.1c). La variation en plasticité est importante d'un point de vue évolutif, car elle souscrit à un des premiers critères nécessaires pour évoluer : la variabilité. Si la plasticité comporte aussi une base génétique, qu'elle est héritable et qu'elle est sous sélection, c'est-à-dire que la variabilité a des conséquences sur la valeur adaptative des individus (Darwin, 1859 ; Merilä et Hendry, 2014), elle peut donc évoluer (Nussey, 2005).

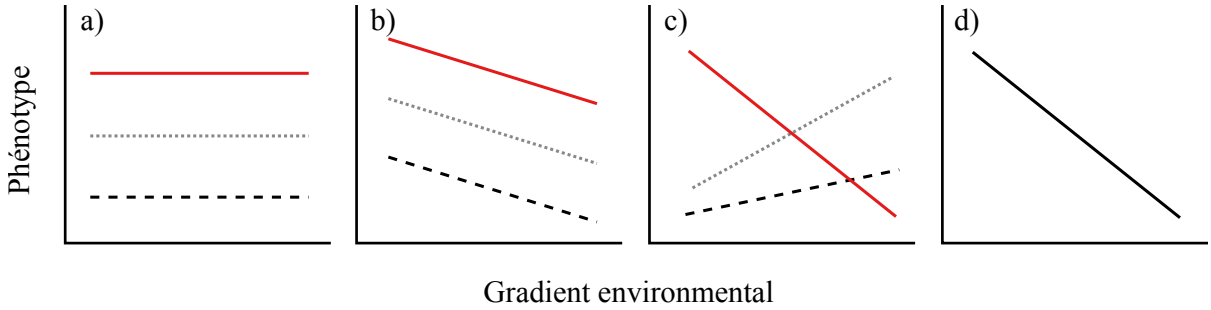


**Figure 1.1 Différents scénarios de plasticité phénotypique, où les normes de réactions représentent différents types d'individus dans une population.**

Dans la figure a), il n'y a pas de plasticité et les individus varient seulement en élévation (intercepte) ; en b) il y a présence de plasticité individuelle, mais les individus varient seulement en élévation et non en pente ; en c), les individus varient à la fois en pente et en élévation et en d) la plasticité existe mais tous les individus ont des pentes et des interceptes identiques.

En contrepartie, la plasticité peut être coûteuse (DeWitt et al., 1998) car elle implique d'« entretenir » les systèmes sensoriels qui permettent de percevoir les changements dans l'environnement (DeWitt et al., 1998 ; Auld et al., 2010). La plasticité peut aussi parfois être limitée par des contraintes, par exemple les conditions à la naissance (Nussey et al., 2005). Aussi, une des difficultés pour mesurer la plasticité phénotypique est d'identifier les déterminants environnementaux qui génèrent une réponse biologique (van de Pol et al., 2016). Des indices liés au climat, tel que le réchauffement des océans (Gibbin et al., 2017) et des variables météorologiques locales (Bourret et al., 2015), ont été utilisés pour étudier la plasticité phénotypique ainsi que les adaptations des organismes (Siepielski et al., 2017). Les facteurs écologiques responsables des réponses plastiques restent toutefois inconnus pour la plupart des mammifères. Même lorsque les signaux environnementaux peuvent être liés à la plasticité phénotypique, les mécanismes sous-jacents aux changements de traits restent souvent obscurs (Uller et al., 2013 ; Forsman, 2015).

### 1.3.3 La microévolution

Si la plasticité phénotypique est un mécanisme d'ajustement à court terme, la microévolution peut être nécessaire à plus long terme et pour faire face aux changements environnementaux plus importants en force et direction (Boutin et Lane, 2014). La microévolution est un méca-

nisme proposé pour expliquer la persistence d'espèces face aux changements climatiques, qui entraînent de nouvelles pressions de sélection sur les populations (Hoffmann et Sgrò, 2011). Ce mécanisme permet aux espèces de s'adapter par des modifications génétiques (Gienapp et al., 2008 ; Merilä et Hendry, 2014). Bien qu'elles puissent être rapides, les réponses microévolutives sont généralement plus lentes que les réponses plastiques, car l'héritabilité des traits phénotypiques dans une population naturelle est souvent modérée, et que plusieurs générations sont nécessaires avant que les changements génétiques ne soient exprimés (Kruuk, 2004). Dans les populations sauvages, les réponses évolutives expliquent moins fréquemment les changements phénotypiques que les réponses plastiques (Merilä et Hendry, 2014 ; Pigeon et al., 2017). D'une part, il est difficile de cibler la force sélective (e.g., le réchauffement climatique), et d'autre part, il est difficile de montrer un changement génétique résultant de cette force (Merilä et Hendry, 2014). Détecter des réponses microévolutives nécessite donc des informations sur l'apparentement entre individus, par exemple un pédigrée, sur plusieurs générations (Gienapp et al., 2008).

## 1.4 Le paradigme de la synchronie

### 1.4.1 Le moment de la naissance

Le moment de la naissance est crucial pour bien des organismes, incluant les mammifères sauvages et les humains (Lummaa et al., 2002). Un mauvais départ, tel un déficit nutritionnel avant ou immédiatement après la naissance, peut être très difficile à rattraper ou alors, implique des coûts à plus long terme (Metcalfe et Monaghan, 2001 ; Marcil-Ferland et al., 2013). Les différences en masse moyenne à la naissance, par exemple, sont si importantes qu'elles influencent les différences inter-cohortes en survie néonatale, le développement à des âges spécifiques et l'âge à la première reproduction chez les cerfs (Albon et al., 1987). Les cohortes de femelles caractérisées par une faible masse à la naissance produisent des faons qui sont plus légers, et la survie des faons nés de mères de cohortes différentes varie de <10% à > 60% (Albon et al., 1987). Suivant les naissances, le développement en bas âge et la période de lactation sont aussi des aspects importants pour la croissance, la survie et le succès reproducteur des mammifères à long terme (Lindström, 1999).

Chez les ongulés, l'environnement maternel est le principal déterminant de la condition du nouveau-né et il inclut la condition corporelle de la femelle à la mise-bas, les conditions écologiques à la mise-bas, la sélection du site de mise-bas, le moment de donner naissance, etc. La date de naissance détermine non-seulement la masse du nouveau-né (Lee et al., 1991) mais chez le mouflon d'Amérique, elle influence indirectement la survie à un an (Feder et al., 2008). Cet effet de la date de naissance sur la survie est indirectement lié à la masse au sevrage des agneaux ; les agneaux nés tard ne survivent pas à leur premier hiver parce qu'ils ne sont pas assez lourds et robustes au moment du sevrage (Feder et al., 2008). À l'opposé, un agneau né tôt a suffisamment de temps pour grandir et prendre de la masse avant son premier hiver, particulièrement rigoureux en milieu tempéré et alpin. La masse est donc un aspect particulièrement important d'un point de vue adaptatif chez cette espèce (Festa-Bianchet et al., 1998) et chez d'autres ongulés (Fairbanks, 1993 ; Côté et Festa-Bianchet, 2001 ; Cook et al., 2004). Cette explication exclut toutefois une compensation partielle d'une date de naissance tardive ou d'une faible masse à la naissance par des soins maternels (i.e., la lactation, abordée plus bas). La qualité et la quantité de végétation disponibles influencent aussi la croissance et la survie des jeunes ongulés (Pettorelli et al., 2007). Il semble toutefois qu'en cas de mauvais départ, un juvénile qui rattraperait sa faible masse en prolongeant sa croissance à l'âge adulte subirait des coûts en succès reproducteur plus tard (Marcil-Ferland et al., 2013).

### 1.4.2 Synchronie et distribution temporelle des naissances

Chez les mammifères, les naissances peuvent suivre un gradient entre une distribution continue, où les naissances ont lieu tout au long de l'année (MacKay et al., 2018), et une distribution définie, où les naissances sont synchronisées les unes aux autres et concentrées sur une courte période (Rutberg, 1987). La distribution des naissances varie de façon générale selon la latitude ; plus la latitude est élevée, plus courte - et synchronisée - sera la saison des naissances (Rutberg, 1987). Toutefois, cette hypothèse générale ne s'applique pas à tous les milieux saisonniers ou tempérés (MacKay et al., 2018). La synchronisation réfère généralement à la distribution temporelle des dates, par exemple, 80% des naissances ont lieu du 30 avril au 27 mai chez les chevreuils, tandis que le *timing* réfère à la date moyenne ou encore, une date de disponibilité maximale de nourriture (Gaillard et al., 1993). On suppose, par exemple, que le moment de donner naissance est optimal lorsqu'il coïncide avec la croissance et l'abondance de végétaux, chez les herbivores, ou avec l'abondance de proies, chez les prédateurs (Both

et al., 2009).

La saisonnalité dans la phénologie de la végétation et la prédation expliquent en partie la distribution temporelle des dates de naissances dans les milieux tempérés et arctiques. Les mères qui mettent bas trop tôt, par exemple, pourraient faire face à un déséquilibre énergétique au début de la lactation (Guinness et al., 1978) ou même compromettre la survie néonatale en exposant davantage les nouveaux-nés à la prédation (Bunnell, 1982). Naître trop tôt pourrait également accroître le risque d'hypothermie ou entraîner une faible masse à la naissance (Fairbanks, 1993 ; Nowak et Poindron, 2006). Les mères qui mettent bas trop tard, à leur tour, ne permettraient pas à leurs jeunes de profiter de la végétation fraiche et de croître suffisamment pour faire face aux conditions hivernales difficiles (Festa-Bianchet, 1988a ; Feder et al., 2008). Les coûts énergétiques liés à certaines phases de la reproduction, comme la lactation, expliquent aussi la distribution temporelle des naissances. Les coûts que représentent la lactation pour les mères et les demandes énergétiques des jeunes détermineront aussi en partie quand la lactation doit-elle débuter et s'arrêter (i.e., le processus du sevrage, voir encadré 2 et la figure 1.2).

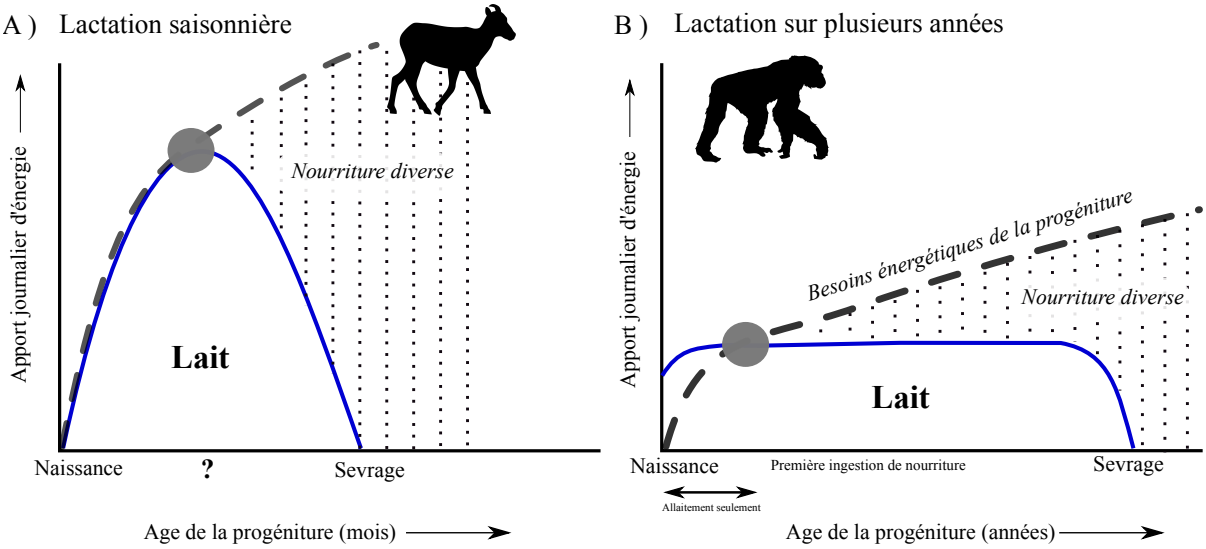


**Figure 1.2 Schéma conceptuel des contributions de la mère et de la progéniture à l'apport énergétique de la progéniture, en relation avec son âge et le moment de la fin de la lactation (indiqué par le sevrage).**

La ligne pleine bleue indique la contribution énergétique de la mère et la ligne pointillée, les besoins de la progéniture. A) Les espèces qui tirent parti de l'abondance saisonnière de nourriture ont une lactation à durée déterminée, comme les petits mammifères, ou encore, le mouflon d'Amérique. Les jeunes s'alimentent par eux-mêmes assez tôt dans leur développement mais ce moment est souvent inconnu, comme illustré par le « ? ». B) Les espèces qui allaitent sur plusieurs années, comme certains primates, ont plutôt un apport d'énergie stable, avec une augmentation progressive de la contribution du nourrisson qui s'alimente de façon indépendante. Les cercles indiquent le moment où le lait est insuffisant pour satisfaire les besoins de la progéniture. Idéalement, ce point coïncide avec le début de l'alimentation indépendante par la progéniture et marque le début du sevrage. La transition entre une alimentation basée sur le lait et une alimentation diverse peut être identifiée au moyen d'isotopes stables (Bădescu et al., 2016). Figure adaptée de Van Noordwijk et al. (2013) et Bădescu et al. (2016).

### 1.4.3 Les soins maternels et la lactation

Chez beaucoup de mammifères, tels les phocidés ou les ongulés, la lactation représente l'essentiel des soins maternels prodigués à la progéniture. La lactation implique la synthèse, la sécrétion et le transfert du lait de la mère vers le jeune (Cowie et al., 1951). C'est un effet

maternel important (Kirkpatrick et Lande, 1989), défini comme l'influence du phénotype de la mère sur celui de sa progéniture, indépendamment du bagage génétique. En influençant la qualité des soins maternels que la progéniture reçoit, l'environnement influence donc indirectement le succès reproducteur à court et à long terme de la progéniture (Mousseau et Fox, 1998). En allaitant, les mères transmettent des nutriments essentiels à leurs jeunes par le lait, principalement en tout début de lactation (Figure 1.2).

D'autres soins maternels ont été observés notamment la communication entre les mères et leur progéniture, le « choix » de partenaires de jeu par la mère, le maintien d'une distance de contact par la mère, etc. (Théoret-Gosselin et al., 2015). Il existe une variation individuelle en soins maternels, et ces soins influencent positivement la survie à l'hiver des ongulés (Théoret-Gosselin et al., 2015), ce qui démontre qu'il existe une diversité de façons avec lesquelles les mères peuvent prendre soin de leur progéniture (Bernardo, 1996). Les observations comportementales permettent aussi de quantifier des aspects importants entourant la lactation tels que la durée et la fréquence des tétées ou encore, le pourcentage de tétées « réussies » par rapport à l'ensemble des tétées et des rejets par les mères (Berger, 1979 ; Festa-Bianchet, 1988b). Bien que ce ne soient pas des proxy reconnus de la quantité de lait prodiguée aux jeunes (Cameron, 1998), ces indices comportementaux montrent une diminution de l'investissement maternel à mesure que la période de sevrage approche (voir l'encadré 2 à ce sujet).

Chez les mammifères qui se reproduisent à chaque année (e.g., les ongulés, les petits mammifères, certains phocidés), le sevrage aura lieu quelques jours à quelques mois suivant les naissances, dans la première année du nouveau-né (Figure 1.2a, Bădescu et al., 2016). Chez d'autres espèces, comme les chimpanzés, les éléphants et les humains, par contre, le sevrage peut prendre plusieurs années (Figure 1.2b, Bădescu et al., 2016). Au sein d'une même espèce, différentes femelles peuvent prolonger la durée des soins maternels, quoique ce prolongement retarde la reproduction suivante (Hogg et al., 1992). La contribution relative des soins maternels et du transfert exclusif de lait jusqu'au moment du sevrage pour expliquer toute la variation en survie en bas âge reste encore inconnue dans la majorité des mammifères sauvages, du moins chez les ongulés. De plus, la variation individuelle dans les stratégies d'allocation des femelles rend encore plus complexe la détermination des niveaux d'investissement maternel au début, au milieu ou à la fin de la période de lactation (Lee et al., 1991).

## Encadré 2. Le processus du sevrage

Le sevrage est la période de transition vers l'indépendance du jeune. Chez certaines espèces d'ongulés, d'otariidés ou de primates, le sevrage est difficile à mesurer puisqu'il s'agit d'un processus étalé sur une longue période, et non d'un moment précis (Berger, 1979 ; Lee et al., 1991). Il se caractérise souvent par une diminution de la longueur et la fréquence des tétées (Berger, 1979 ; Festa-Bianchet, 1988b). Chez d'autres espèces comme les éléphants, le sevrage correspond plutôt au moment où la mère entre à nouveau en oestrus, puisque l'allaitement peut continuer jusqu'à la naissance de la prochaine progéniture (Lee et al., 1991). Une variabilité individuelle à l'étape du sevrage est possible, et les traits maternels comme la masse corporelle influencent la durée de la lactation (Lee et al., 1991). Une augmentation de l'investissement maternel au « moment » du sevrage pourrait se traduire par le prolongement des soins maternels pour un des sexes (Hogg et al., 1992). Selon la sévérité du milieu et de la condition énergétique de la femelle, le sevrage peut débuter lorsque le jeune atteint une masse suffisante (Lee et al., 1991). Ceci augmente les chances de survie au premier hiver, qui représente une période limitante pour beaucoup d'ongulés vivant en milieu tempéré (Berger, 1979 ; Sæther, 1997).

Les dépenses (pour la femelle) et les besoins énergétiques (pour la progéniture et la femelle) pendant la période de lactation détermineront non-seulement le moment de transition vers l'indépendance du jeune, mais ils dicteront aussi le moment de donner naissance. Il peut arriver que les besoins énergétiques de la progéniture après ou pendant le sevrage excèdent ceux de la mère pendant le reste de la période de lactation. Par exemple, la durée de la lactation étant de 6 semaines chez les chauves-souris africaines (Microchiroptera *sp.*), les naissances ont lieu environ un mois avant le moment où les insectes sont les plus abondants, pour que les jeunes sur le point d'être sevrés profitent de cette nourriture abondante (Cumming et Bernard, 1997). La disponibilité de nourriture maximale pourrait ainsi ne pas coïncider seulement avec les naissances mais avec le moment où la transition vers le sevrage est effectuée. Chez les mouflons (Berger, 1979), le sevrage est plutôt une période de conflit puisque les mères sont « contraintes » de se reproduire à nouveau à la fin de la lactation, alors que les jeunes de l'année demandent encore à être allaités. Le sevrage ne semble donc pas coïncider avec une augmentation des ressources alimentaires pour donner ce « coup de pouce » aux jeunes sevrés. Tout compte fait, très peu d'information subsiste quant au niveau relatif d'investissement maternel dans les quelques jours qui suivent la naissance, pendant le « pic » de lactation, ou encore, pendant le processus de sevrage.

### 1.4.4 Coût de la lactation et *timing*

Sur le plan énergétique, la lactation est coûteuse car les mères ont à la fois besoin d'énergie pour subvenir à leurs propres besoins, ainsi qu'à ceux de leur progéniture (Rogowitz, 1996). Lors de la production de lait, les mammifères placentaires, comme les ruminants, augmenteraient leur dépense énergétique jusqu'à 4 fois leur taux métabolique de base (Oftedal, 1985). De plus, contrairement à la gestation, la lactation comporte des désavantages adaptatifs, c'est-à-dire qu'elle diminue les chances de survie et le succès reproducteur futurs des mères (Clutton-Brock et al., 1989). En complément aux stratégies de reproduction, le *timing* des naissances peut optimiser la reproduction en minimisant ses coûts énergétiques. Pour pallier ces coûts, les femelles doivent toutefois être en bonne condition pour assurer la gestation, la naissance et l'allaitement, et certaines doivent moduler le *timing* de la naissance pour que la lactation coïncide avec le pic d'abondance de nourriture au printemps. Ceci est particulièrement vrai chez les espèces à reproduction saisonnière comparativement aux espèces où la lactation se poursuit sur plusieurs années (Figure 1.2, Bădescu et al., 2016).

Chez les ongulés qui vivent en milieu tempéré ou saisonnier, les naissances ont souvent lieu au printemps, supposément pour qu'elles coïncident avec la disponibilité maximale de nourriture. Le moment et la synchronie des naissances sont les conséquences d'un ajustement des dates de conception (Hogg et al., 2017), et parfois d'une flexibilité dans la durée de la gestation (Holand et al., 2006 ; Clements et al., 2011). Une ovulation tardive entraînerait une naissance tardive, et la progéniture née tardivement est plus susceptible de mourir au cours de son premier hiver (Feder et al., 2008). Les soins maternels peuvent augmenter les chances de survie de la progéniture, mais la prolongation des soins maternels peut aussi réduire les reproductions futures de la mère (Hogg et al., 1992). Un moment propice aux naissances devrait favoriser la survie des juvéniles grâce à un lait de qualité, et à une végétation abondante et en croissance pour les mères et les jeunes. Le début de la lactation devrait être synchronisé avec la période optimale de disponibilité de nourriture pour permettre aux mères de soutenir ce coûteux transfert d'énergie. Or, étonnamment peu d'études en milieu sauvage testent réellement cette supposition. Les herbivores vivant en milieux tempérés pourraient adopter des stratégies intermédiaires entre les stratégies « capitaliste » et « journalière » (Hamel et Côté, 2009). À plus fine échelle, les jeunes femelles modifieraient même leur comportement d'alimentation pour répondre à la fois aux exigences énergétiques de la lactation et à leur propre croissance (Blanchard, 2005 ; Hamel et Côté, 2009), surtout si les coûts liés à la lactation changent au cours d'une même saison

(Hamel et Côté, 2009). La modulation de la composition du lait représente l'intégration la plus fine de changements qui peuvent survenir dans l'environnement, et donc, affecter l'individu et sa physiologie.

### 1.4.5 La composition du lait

La composition du lait fait est rarement liée aux paramètres de succès reproducteurs des mammifères sauvages, bien qu'elle soit décrite chez plusieurs espèces (Oftedal, 1984). La variation en composition du lait s'est montrée déterminante pour la survie de grands (Quesnel et al., 2017) et petits mammifères (Skibiel et Hood, 2015), et est corrélée au gain de masse des chiots chez le phoque gris (*Halichoerus grypus*, Lang et al. (2009)). De plus, la composition du lait peut être 1) répétable pour une même femelle (Lang et al., 2009), 2) influencée par plusieurs sources de variabilité, comme l'âge du jeune (Lang et al., 2005 ; Renaud et al., 2019) ou de la mère (Lang et al., 2009), les gènes (Sanna et al., 1997) ou l'environnement (Quesnel et al., 2017), et 3) modulée de façon complexe chez certaines espèces notamment les marsupiaux (Trott et al., 2002 ; Robert et Braun, 2012). La composition du lait représente donc un élément important des soins maternels chez les mammifères et influence forcément des traits d'histoire de vie en bas âge.

La composition du lait peut être caractérisée par les macronutriments, c'est-à-dire les protéines, les gras et les sucres comme le lactose ou le glucose. La majorité de l'énergie transmise par le lait est contenue dans son gras (Oftedal, 1984). Des études sur les animaux captifs et domestiques montrent aussi l'importance des minéraux du lait, ou micronutriments, qui, en combinaison avec les immunoglobulines, les enzymes et les hormones, améliorent l'immunité du jeune, sa croissance et son développement (Hamosh, 2001 ; Gallego et al., 2009). Chez le cerf élaphe (*Cervus elaphus hispanicus*), les concentrations en calcium, phosphore et fer du lait sont déterminantes pour la croissance des faons, et cette concentration varie selon le sexe du faon (Gallego et al., 2009). Le lait destiné aux mâles a une teneur plus faible en calcium et phosphore et une plus grande teneur en potassium et magnésium que le lait destiné aux femelles (Gallego et al., 2009). Les minéraux interagissent aussi avec d'autres enzymes et minéraux pour assurer les fonctions métaboliques et catalytiques (Cashman, 2006). Par exemple, le sodium et le potassium, en combinaison, aident à l'absorption du glucose dans l'organisme (Barboza et al., 2009). De façon générale, la combinaison de calcium et de phosphore dans le

lait est cruciale à la formation du squelette et des muscles (Barboza et al., 2009).

Chez le phoque gris, la concentration en acides gras du lait provient principalement des réserves maternelles (Lang et al., 2009). Toutefois, une partie importante de la variation en composition du lait n'est pas expliquée par les différences individuelles en masse corporelle (37% de la variation) ou en réserves maternelles de gras ou protéines (43% et 27% de la variation) (Mellish et al., 1999). Une partie de la variation en composition du lait est donc spécifique à chaque femelle et à sa capacité physiologique de moduler la quantité d'énergie qu'elle transfère à sa progéniture par le lait (répétabilité de 0,38 pour le % de protéines et 0,50 pour le % de gras du lait ) (Lang et al., 2009).

Outre les différences individuelles et les traits maternels, la variabilité environnementale explique aussi la variation en composition du lait en affectant la quantité ou la qualité de nourriture disponible pour les mères. Une alimentation de faible qualité diminue la concentration de gras, protéines et lactose du lait chez les cerfs élaphes (Landete-Castillejos et al., 2003). Ce changement devient apparent à la quatrième semaine de lactation ; avant la quatrième semaine, les femelles moins bien nourries semblent plutôt capables d'augmenter la concentration de gras du lait pour compenser leur faible volume de lait. Les réserves corporelles seraient alors utilisées pour compenser le manque de nourriture (Landete-Castillejos et al., 2003). La variation de la composition du lait peut donc refléter les réserves maternelles (Wohlt et al., 1981) ou l'apport journalier de nourriture (e.g., le profil d'acides gras, Coppa et al. (2015)).

Pour résumer, un élément clé des effets maternels est leur plasticité. Les réponses populationnelles et individuelles observées suggèrent que les organismes ont des mécanismes pour s'ajuster aux changements de leur environnement. Toutefois, de nombreuses questions demeurent en suspens. Quels sont les traits, les déterminants environnementaux et les moments de l'année qui permettent aux organismes de se re-synchroniser avec leur nourriture, par exemple chez les espèces longévives ? Les espèces à stratégie « capitaliste » répondent-elles aussi aux changements phénologiques ? Comment tester la notion de « *match-mismatch* » en milieu naturel ? Quelles sont les conséquences sur la valeur adaptative ?

## 1.5 Objectifs

L'objectif global de mon doctorat est de clarifier les liens entre les traits liés à la reproduction et les variations phénologiques chez les brebis du mouflon d'Amérique. Je m'attarderai principalement aux dates de mise-bas, à la mortalité néonatale et à la composition du lait.

De cette question globale découlent trois chapitres qui visent à :

1. Déterminer la présence de plasticité phénotypique en dates de naissance en réponse aux variations de climat et conditions météorologiques locales ;
2. Quantifier l'importance relative des différences annuelles et individuelles dans la composition du lait maternel ;
3. Quantifier l'impact de la variation en phénologie de la végétation sur la survie néonatale, la composition du lait, et les conséquences de cette variation sur l'agneau.

Répondre à ces objectifs demande un suivi à long terme individuel très détaillé. Les mouflons d'Amérique *Ovis canadensis* de Ram Mountain sont marqués et suivis de la naissance à la mort depuis 1971, et dans mon étude, plusieurs captures par individu ont été essentielles pour déterminer la présence de plasticité phénotypique. La généalogie de la population a été reconstruite ce qui permet d'assigner les maternités et les paternités, et de quantifier l'importance relative des changements génotypiques et plastiques dans les changements de traits. De plus, des mesures morphologiques sont prises annuellement, et dans les six dernières années, nous avons pu collecter des échantillons de lait, ce qui n'est pas toujours possible en milieu naturel. Pour mesurer la phénologie de la végétation, j'ai utilisé plusieurs indices provenant de sources différentes. Notamment, j'ai eu accès aux indices dérivés d'images satellitaires, qui m'ont permis de travailler tant avec la durée de la saison végétative qu'avec des dates d'émergence des plantes au printemps. Toutes ces données combinées m'ont fourni une mine d'information qui a permis la combinaison d'analyses aussi bien phénotypiques et physiologiques nécessaires pour atteindre mes objectifs.

## 1.6 Méthodes

### 1.6.1 Espèce étudiée

Le mouflon d'Amérique est un ongulé présent dans les habitats alpins des Rocheuses jusque dans les déserts du nord du Mexique (Figure 1.3). Au Canada, l'espèce est répartie dans les montagnes Rocheuses de l'Alberta et de la Colombie-Britannique. Les mouflons d'Amérique sont sexuellement dimorphiques, les mâles ayant une plus grande masse et de longues cornes (Shackleton et Haywood, 1985) ; les mouflons présentent un système de reproduction polygyne. En dehors du rut, les femelles, les agneaux et les jeunes mâles forment des groupes de «pouponnières» tandis que les mâles matures forment des groupes séparés (Ruckstuhl, 1998). Le rut commence à la fin novembre. La gestation dure environ 175 jours et les brebis donnent naissance à un seul agneau. Les femelles peuvent être sexuellement matures à 18 mois bien que les femelles de notre zone d'étude aient leur premier agneau à 3 ans en moyenne. La plupart des agneaux naissent autour du 30 mai, mais des naissances tardives ont été observées. La lactation dure environ jusqu'à la fin du mois de septembre ou octobre, mais il est difficile d'estimer exactement la date du sevrage. Celui-ci pourrait avoir lieu lorsque les agneaux ont entre 120 et 150 jours, mais certaines femelles sèvrent leur agneau en octobre (Festa-Bianchet, 1988b). La plupart des femelles sexuellement matures conçoivent à nouveau lors du prochain oestrus. La sénescence reproductive commence à l'âge de 13 ans (Festa-Bianchet et King, 2007), alors que la sénescence en survie et en masse corporelle commence à l'âge de 11 ans (Berube et al., 1999). Peu de femelles vivent plus de 15 ans, alors que les mâles survivent rarement plus de 12 ans (Loison et al., 1999).

La masse corporelle est un trait important chez le mouflon d'Amérique et affecte à la fois la survie et la reproduction (Festa-Bianchet, 1998). En général, une masse importante est associée à une meilleure probabilité de survie surtout chez les agneaux et les juvéniles (Festa-Bianchet et al., 1997). À un an, les femelles légères ont moins de chances de survie que les femelles plus lourdes. La survie des mâles de 1 an et des mâles adultes semble indépendante de la masse (Festa-Bianchet et al., 1997) mais la masse est liée au rang social et au succès reproducteur des béliers (Pelletier et Festa-Bianchet, 2006). L'accumulation de réserves de graisse permet aux brebis de supporter les coûts de la reproduction ; les mouflons sont considérés comme des reproducteurs par « capital » (Festa-Bianchet, 1998). La condition physique, souvent mesurée

par la masse corporelle, est un aspect essentiel de la reproduction. Les brebis sevrant un jeune sont en général 1,5% plus lourdes que celles ne sevrant pas leur agneau (Festa-Bianchet, 1998). La taille de population (i.e., densité) est un autre déterminant important de la reproduction et de la survie. À haute densité, les brebis plus lourdes ont un meilleur succès reproducteur que les femelles plus légères (Festa-Bianchet et al., 1998) et la probabilité de survie des agneaux diminue avec la densité à la naissance (Festa-Bianchet et al., 1998). Jorgenson et al. (1997) ont montré que la densité semblait également affecter la survie des femelles d'un an. Lorsque la taille de la population augmente, les femelles emploient une tactique de reproduction «égoïste» et réduisent leur effort de reproduction, ce qui entraîne une baisse de la survie hivernale de leurs agneaux. Les femelles favorisent ainsi leur condition physique au détriment de celle de leurs agneaux lorsque les ressources sont limitées (Martin et Festa-Bianchet, 2010).

### 1.6.2 Socialité chez le mouflon d'Amérique

De façon générale, la socialité chez le mouflon d'Amérique suit une dynamique de fission-fusion (Vander Wal et al., 2015). L'espèce est grégaire et dans la population de Ram Mountain, la taille de groupe moyenne est d'environ 20 individus (Vander Wal et al., 2015). La composition des groupes est notée tous les jours sur le terrain. Elle est assez fluide et varie aux 2-3 jours (L'Heureux et al., 1995), soit par l'arrivée ou le départ d'individus d'autres groupes. Les femelles, leur agneau, les juvéniles mâles et femelles et les jeunes mâles de moins de 2-3 ans forment habituellement l'essentiel d'un groupe de « pouponnières ». Les mâles adultes se dispersent, forment des groupes séparés et sont rarement vus pendant l'été (Cassirer et al., 2013 ; Vander Wal et al., 2015). Ces mâles, aux histoires de vie différentes, rejoindront les femelles au moment du rut, à l'automne (Vander Wal et al., 2015).

Les groupes sont organisés de façon hiérarchiques (Vander Wal et al., 2015). Les femelles les plus âgées (et les plus lourdes) sont souvent les plus dominantes et guideront un groupe (Vander Wal et al., 2015), qu'il soit stable ou non. La centralité, i.e., le nombre d'associations directes et indirectes d'un individu dans un réseau social, d'une même femelle au sein d'une groupe est répétable (r= 0,4) et la centralité d'une femelle augmente sa probabilité de produire un agneau et de survie à l'âge adulte (Vander Wal et al., 2015). La centralité est aussi importante que la masse ou la densité de population en terme de valeur adaptative des femelles (Vander Wal et al., 2015). Les liens sociaux sont donc importants même s'ils n'influencent pas directement

la survie des agneaux.

Les femelles et leur agneau sont habituellement à proximité physique, du moins dans le même groupe d'individus. Il peut arriver, toutefois, que certaines femelles « gardent » les agneaux d'autres femelles en leur absence, temporairement. Le principal soin maternel prodigué aux agneaux est l'allaitement (Oftedal, 1985 ; Festa-Bianchet, 1988b). Les comportements maternels associés à l'allaitement (contact physique, maintien d'une distance de proximité, etc.) peuvent indirectement améliorer la survie des agneaux en diminuant notamment le risque de prédation (Shackleton et Haywood, 1985) et en renforçant le lien mère-agneau, essentiel au développement du jeune (Nowak, 2000 ; Spinka et al., 2001). Sauf ces associations mères-agneaux, les mouflons forment peu d'associations avec des individus apparentés (Vander Wal et al., 2016). Les femelles forment toutefois des associations non-aléatoires avec d'autres femelles de statut reproducteur similaire (Vander Wal et al., 2016).

### 1.6.3 Aire d'étude et récolte d'échantillons

La population de mouflons d'Amérique est suivie depuis plus de 40 ans. Ram Mountain, en Alberta (52°N, 115°W, altitude de 1080 à 2170 m), est un massif montagneux situé à 30 km à l'est de la chaîne principale des Rocheuses canadiennes. Les forêts alpines et subalpines caractérisent la région et les mouflons utilisent environ 38 $km^2$ de cet habitat. L'immigration et l'émigration sont rares, en partie à cause de barrières naturelles comme la rivière North Saskatchewan au Nord de l'aire d'étude.

Le suivi à long terme de la population a commencé en 1971 et se poursuit chaque année de la fin mai à la fin septembre. Les mouflons sont capturés à l'aide d'une trappe de type « corral » appâtée avec du sel. La trappe a deux portes indépendantes qui peuvent être fermées à distance, permettant aux trappeurs d'attendre discrètement que les individus voulus soient capturés. À chaque capture d'individu, des mesures morphologiques (masse et taille des cornes) sont prises. Les animaux sont capturés une fois entrés à l'extérieur de la trappe, masqués, trois de leurs pattes attachées, puis sortis à l'extérieur pour la prise de mesures. Aucun tranquillisant n'est utilisé pendant la manipulation. Tous les nouveaux individus sont marqués avec des identifiants uniques ; les femelles sont identifiées avec des colliers de couleur et de motif uniques, tandis que les mâles sont identifiés avec une combinaison unique d'étiquettes d'oreilles. Les agneaux

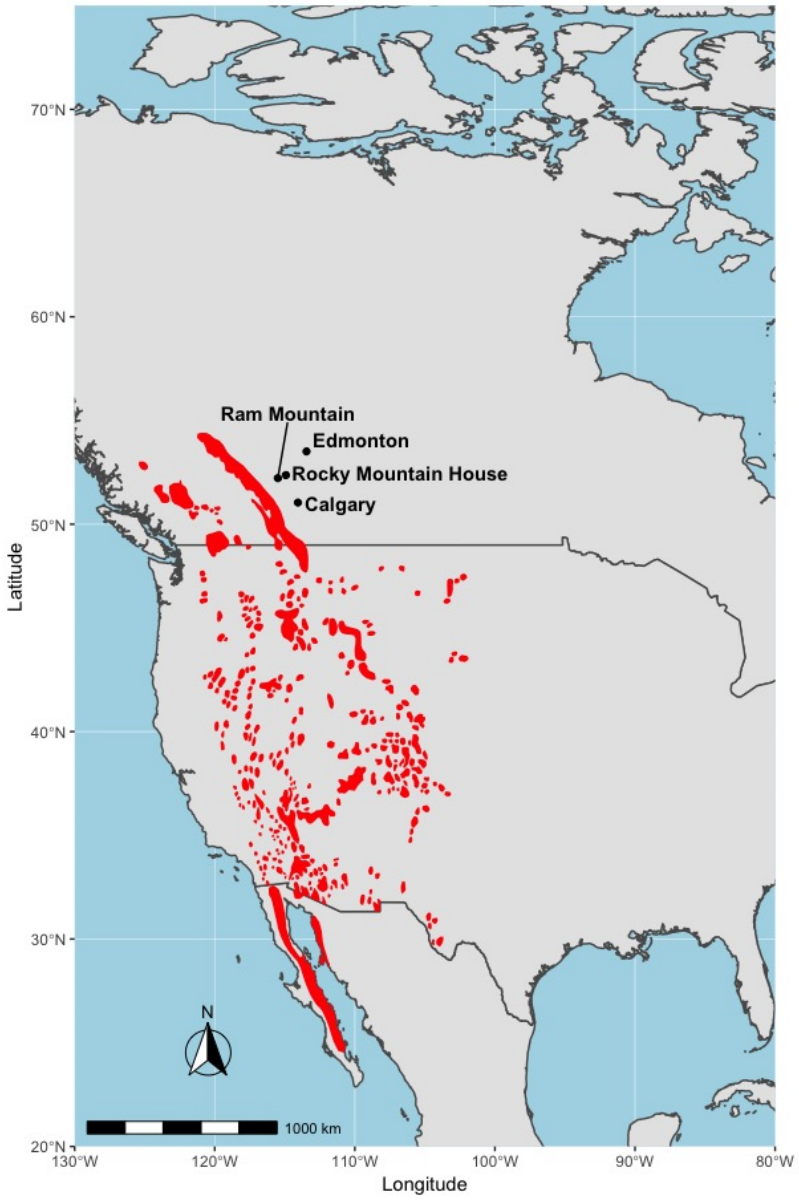


**Figure 1.3 Distribution du mouflon d'Amérique. En rouge sont les localisations répertoriées par l'IUCN.**

sont marqués d'une étiquette d'oreille en métal de type « Ketchum » attachée à un ruban de plastique coloré. La plupart des agneaux sont marqués au cours de leur premier été et plus de 98% de la population est marquée ; la taille exacte de population et la structure par âge sont connues chaque année.

Les animaux peuvent être pesés au moyen d'une balance accrochée à un système de poulie. Les cornes des mâles et des femelles sont mesurées par la suite, à l'aide d'un ruban à mesurer flexible. La largeur et la forme des cornes des mâles sera notée, et la croissance de l'année en cours sera notée pour les mâles et les femelles. Entre 2011 et 2016, des échantillons de lait ont aussi été récoltés. Cette récolte d'échantillon avait lieu en même temps que les manipulations « de routine », c'est-à-dire suivant un échéancier de capture d'environ aux 21 jours (pour les brebis) pour éviter d'infliger trop de stress aux animaux. Une fois la femelle couchée au sol pour les manipulations, une pression du pis permettait d'en extraire le lait (voir la figure 1.4) dans un tube de plastique identifié et daté. Les échantillons étaient placés au congélateur jusqu'au moment de les expédier au laboratoire de l'Université de Sherbrooke. En moyenne, les agneaux, les femelles adultes et les mâles adultes sont capturés respectivement 2, 3,5 et 2,6 fois par année. Les mesures répétées de masse sont utilisées pour déterminer la masse estimée au 5 juin et au 15 septembre à l'aide de la méthode proposée par Martin et Pelletier (2011).

Le statut reproducteur des brebis est déterminé par examen du pis. C'est notamment grâce à cette méthode qu'on peut déterminer si une mortalité néonatale explique l'absence d'un agneau aux côtés d'une brebis. L'examen du pis, selon le moment de la capture au cours de la saison, nous indique que la brebis est enceinte (présence de colostrum), allaitante avec agneau, allaitante mais aucun agneau n'a été vu (mortalité néonatale), n'a pas de trace de lait (ne s'est pas reproduite) ou ne produit plus de lait après en avoir produit ou après avoir été vue allaitant un agneau (potentiellement à cause d'une mortalité estivale ou d'un abandon). L'observation des interactions brebis-agneaux tout au long de la saison permet aussi de confirmer l'identité des mères. Le succès reproducteur des femelles est donc connu depuis 1975 et celui des mâles depuis 1988, par l'attribution génétique des paternités.

Le pédigrée de la population a été reconstruit. Depuis le début de l'étude, les maternités sont assignées à l'aide d'observations comportementales des duos mère-agneau. Depuis 1988, des échantillons de tissus des oreilles sont prélevés sur tous les individus pour faire des assignations génétiques de paternité basée sur 26 loci microsatellite, nous permettant de reconstituer une généalogie où 945 maternités et 608 paternités ont été assignées (Coltman et al., 2005).

**Figure 1.4 Récolte d'un échantillon de lait sur une brebis du mouflon d'Amérique, Ram Mountain, Alberta, Canada**

CHAPITRE 2

# LA PLASTICITÉ PHÉNOTYPIQUE EN DATES DE MISE-BAS

*Every individual matters. Every individual has a role to play. Every individual makes a difference.* Jane Goodall

## 2.1 Description de l'article et contribution

Dans un contexte de changements climatiques, l'environnement peut être variable et imprévisible, et les organismes doivent ajuster leur cycle de reproduction pour qu'il soit toujours optimal. Une façon de s'ajuster est par la plasticité phénotypique (l'autre étant par la microévolution ou encore la migration) ; elle est définie par l'expression de plusieurs phénotypes par un seul génotype, ou individu. À Ram Mountain, nous avons remarqué que les dates de naissance médianes des agneaux ont été devancées d'une dizaine de jours en 26 ans de suivi. Nous avons posé l'hypothèse que cette tendance populationnelle se reflète aussi à une échelle individuelle, celle de la plasticité phénotypique, en réponse aux changements environnementaux qui ont eu lieu dans notre site d'étude. L'article visait à 1) mesurer les déterminants de la variation en dates de mise-bas à l'échelle de la population et 2) quantifier la présence de plasticité phénotypique en dates de mise-bas en réponse aux variables déterminantes à l'échelle populationnelle. Nos résultats montrent principalement des réponses au niveau populationnel, et une réponse individuelle pour les précipitations.

Pour cet article, j'ai participé à l'élaboration des analyses à effectuer avec Fanie Pelletier, Gabriel Pigeon et Marco Festa-Bianchet. J'ai ensuite effectué les analyses statistiques et l'écriture de la première version du manuscrit. Gabriel Pigeon à contribué aux analyses de plasticité. Fanie Pelletier et Marco Festa-Bianchet ont par la suite commenté plusieurs versions du manuscrit, et ont contribué à l'interprétation des données et à la révision du manuscrit. J'ai participé aux travaux de terrain et la collecte de données durant trois saisons, de 2014 à 2016.

**Phenotypic plasticity in bighorn sheep reproductive phenology : from individual to population**

*Behavioral Ecology and Sociobiology* (2019) 73(4) : 1 :13.

Limoilou-Amelie Renaud, Gabriel Pigeon, Marco Festa-Bianchet et Fanie Pelletier

## 2.2 Abstract

Climate change can lead to a mismatch between resource availability and key life history events. Without plasticity in reproductive traits, that mismatch can lower fitness and decrease population size. In birds, phenotypic plasticity is frequently reported as the main mechanism to track environmental changes, but evidence for plasticity in large mammals is scarce. Using long-term individual-based data, we quantified phenotypic plasticity in 394 parturition dates of 137 bighorn sheep ewes (average 2.9 per ewe, range 1–11 parturition dates) in response to environmental drivers. Over 26 years, we detected a population response to environmental drivers, as median parturition date advanced by 15 days. Our study area showed temporal trends in population density, precipitation in October–November and temperature in August–November. Increasing autumn precipitation was associated with later parturition. Increasing autumn temperature was associated with earlier parturition but the effect was weak. Analyses of the between- and within-individual components of weather, climate and density revealed an individual adjustment to autumn precipitation. We detected no plasticity in response to variation in temperature and density and no variation in plasticity (no $I \times E$) in response to any determinants of parturition date. Our results suggest that the reproductive phenology of species with long and fixed gestation may respond more to environmental drivers in autumn than in spring.

## 2.3 Significance statement

Many organisms time reproductive events based on seasonal availability of food resources. Climate change, however, can affect the timing of food availability. Organisms can change the timing of reproduction over the short term through phenotypic plasticity. Little is known, however, about how much individual plasticity in reproductive timing exists in wild mammals. We examined phenotypic plasticity in parturition date in bighorn sheep in response to changes in autumn precipitation, autumn temperature, a global climate index and adult female density. Temporal trends in these variables over 26 years partly explained a 15-day advance in average parturition date. Individual ewes only appeared to show plasticity in response to autumn precipitation, suggesting some capacity to cope with rapid global environmental changes over the short term.

**Keyword :** Climate change, Large vertebrate, Parturition date, Phenotypic plasticity, Precipitation, Temperature

## 2.4 Introduction

Rapidly changing climate presents organisms with the challenge of synchronizing reproductive phenology with temporally shifting availability of seasonal resources (Parmesan, 2006). Phenological traits are especially sensitive to rapid environmental changes because even slight mismatches in timing, for example of parturition or laying date, can compromise juvenile survival (Both et al., 2009; Ozgul et al., 2010). By altering the timing of phenological events, particularly the onset of spring in temperate and boreal environments, changes in climate modify the temporal windows to which organisms must synchronize reproduction. Although some species respond by changing reproductive timing, including parturition date (Ozgul et al., 2010; Moyes et al., 2011), negative fitness consequences of phenological mismatch have been reported, such as delayed emergence of hibernators leading to inability to accumulate sufficient fat before the next hibernation (Lane et al., 2012). Synchrony of reproductive traits and resource availability is important for demography because it may affect offspring growth and survival (Ozgul et al., 2010; Plard et al., 2014), especially in environments with a short season when high-quality food resources are available (Visser et al., 2012).

Organisms can adapt to climate change either through phenotypic plasticity (Charmantier et al., 2008; Porlier et al., 2012) or microevolution (Boutin and Lane, 2014; Merilä and Hendry, 2014). For example, individual phenotypic plasticity allows great tits (*Parus major*) to adjust laying date in response to increasing spring temperature without microevolution, enabling the population to closely track a rapidly changing environment (Charmantier and Gienapp, 2014). Phenotypic plasticity is generally accepted as the main mechanism to cope with short-term changes in the environment, especially in birds (reviewed in Gienapp et al., 2008), but microevolution may be required over a longer time scale if changes increase in magnitude (Boutin and Lane, 2014).

One challenge in measuring phenotypic plasticity is establishing which environmental predictors are most likely to affect phenology (van de Pol et al., 2016). Both climate-related indices, such as ocean warming (Gibbin et al., 2017) and local weather variables such as mean temperature (Bourret et al., 2015) and precipitation (Hogg et al., 2017; Siepielski et al., 2017) have been used to investigate phenotypic plasticity as well as adaptations (Siepielski et al., 2017). The ecological drivers of plastic traits, however, remain unknown for most mammals. Even when environmental cues can be linked to plastic variation in traits, mechanisms underlying

trait responses often remain unclear (Uller et al., 2013 ; Forsman, 2015).

Demographic parameters can also influence the expression of phenotypic plasticity in reproductive traits. In moose (*Alces alces*), population density, winter climate and age affect the timing of conception (Veeroja et al., 2013). Density-dependence in timing of reproduction can be driven by changes in body condition caused by competition for resources (Mysterud et al., 2008), but density effects are not always consistent. Following changes in temperature, female swallows (*Tachycineta bicolor*) expressed less phenotypic plasticity in laying date at low than at high density (Bourret et al., 2015), likely because low density was associated with less suitable habitat. Red squirrels (*Tamiasciurus hudsonicus*), on the other hand, are territorial and breeder density is related to competition for resources (Dantzer et al., 2013). High density leads to increased maternal glucocorticoids in gestating females. Increased hormonal concentrations, here a plastic trait, are transmitted to offspring to 'prepare' for the expected future environment ; this hormonal increase leads to higher growth rate in young squirrels through maternal phenotypic plasticity (Dantzer et al., 2013). Multiple ecological variables can have synergetic effects on phenological traits, complexifying the choice of relevant environmental variables at the population and individual levels.

Both environmental and maternal conditions during the rut affect parturition date in bighorn sheep (*Ovis canadensis*) at the population level (Feder et al., 2008), but it remains unknown if individuals show variability in plastic responses to environmental changes. The first objective of this study was to investigate environmental drivers of variation in parturition date in bighorn sheep at the population level. Bighorn sheep show very little flexibility in gestation length ($\overline{x} \pm SD = 172.7 \pm 2.1$ days ; Hogg et al. 2017) ; we thus hypothesized that phenological changes near the time of conception the previous autumn would affect parturition date. Changes in parturition date could be explained by changes in weather and climate variables or in population density, or both. Density sharply decreased during our study, potentially relaxing competition for forage and favouring earlier parturition (Festa-Bianchet, 1988a ; Pigeon et al., 2017). In parallel, our study area experienced a substantial increase in autumn temperature and a decrease in precipitation. Increasing autumn temperatures, combined with dry conditions, may be followed by rapidly shifting plant phenology and seasonal availabilities of food resources (Post and Stenseth, 1999) – perhaps extending the season of plant growth and therefore improving female condition during autumn. We therefore expected that higher temperatures and higher precipitation in autumn would respectively advance and delay parturition

date at the population level. In the study population, high density is associated with later parturition date (Rioux-Paquette et al., 2011) but whether individuals show variation in their plastic response to density is unknown. We expected that increasing density (number of females aged ($\geq$ 2 years) at conception would delay parturition because of density-dependent competition for resources.

Our second objective was to identify the drivers of individual-level phenotypic plasticity in parturition date. To quantify individual differences in parturition date, we calculated repeatability of parturition date using linear-mixed-effects models (Nakagawa and Schielzeth, 2010). Plasticity can be approximated as the 'reverse' of repeatability (1-R ; Nakagawa and Schielzeth, 2010). Repeatability can be low for two reasons : high within-individual (residual) variation or low between-individual variation. In our study, repeated measurements of parturition date were taken in different environments (years) ; environmental and 'individual-by-environment interaction' (or $I \times E$ ; Nussey et al., 2007) variance components contribute to the residual variance and, thus, to 1-R (Nakagawa and Schielzeth, 2010). Despite potential between-individual differences in parturition date, we expected repeatability to be low because of high residual variance, leaving high potential for phenotypic plasticity in parturition date. Thus, we hypothesized that the population response to weather, climate and density was driven by individual plasticity. Distinguishing between average population responses from individual-level phenotypic plasticity can provide important insights on whether or not long-lived animals can express rapid responses to environmental changes. Individuals may also differ in their degree of plasticity ($I \times E$ ; Nussey et al., 2007). We thus quantified variation in individual plasticity in parturition date. We used long-term data from a wild population of bighorn sheep in Alberta, Canada, with repeated measurements of individuals from birth to death. We first quantified temporal trends in average parturition date over 26 years and the influence of environmental and demographic variables at the population level using a linear mixed model framework. We then used mean-centering (van de Pol and Wright, 2009) to investigate plastic responses to ecological variables.

## 2.5 Methods

### 2.5.1 Study area and data collection

The Ram Mountain bighorn sheep population is located in Alberta, Canada (52°N, 115°W, elevation 1080–2170 m). Since 1971, sheep have been captured in a corral trap baited with salt and monitored daily from late May to late September. Parturition date was estimated from 1992 to 2017 (except for 1993) by a combination of behavioural and morphological observations, including lamb size, presence of the umbilical cord and dark grey coat (Geist, 1971). Ewes isolate themselves for 2–3 days post-partum with their lambs before they join other ewes (Festa-Bianchet, 1988b). Numbers of days since the ewe was last seen before giving birth allowed us to estimate age of lambs and to backtrack their birthdate. We recorded parturition date in Julian day starting 1 January.Weaning is in late September (Festa-Bianchet, 1988b).To meet normality assumptions, we log-transformed parturition date starting with Julian day 136 as day 0 (i.e. the earliest estimated parturition date ; Fig. 2.1). Behavioural observations during lactation and molecular analyses confirmed mother–young associations. Since our study involved marked animals in the field, it was not possible to record data blind.

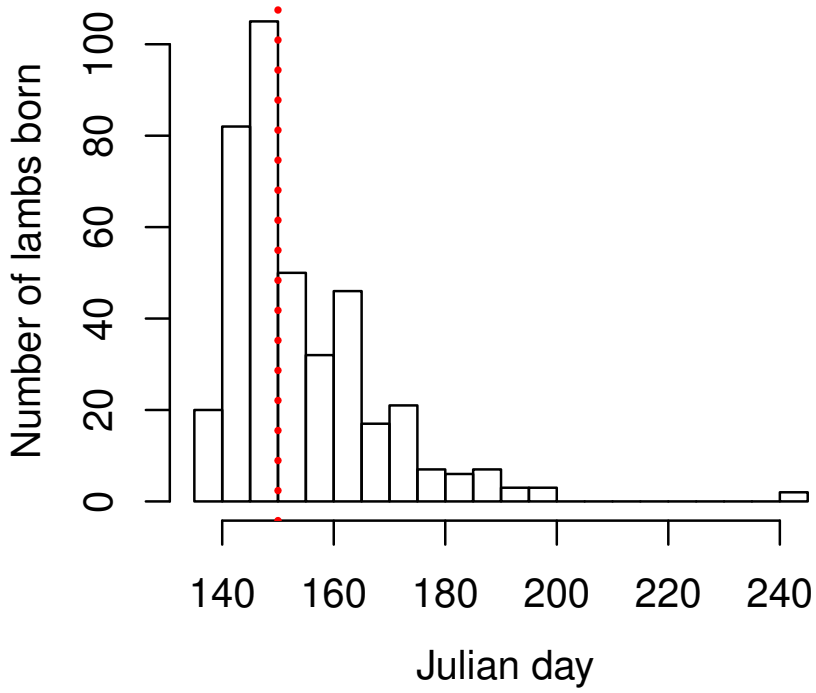


**Figure 2.1 Bighorn lambs born each Julian day at Ram Mountain, Alberta, Canada, 1992 to 2017.**

The median birthdate of May 30th is indicated by a vertical dotted line (non-leap year)

Of all lambs that survived to mid-September, 98% were marked in their first summer (see Jorgenson et al. (1993) for details on sampling design). From 1992 to 2017, 137 females gave birth at least once (average 2.9 times, range 1–11 parturition dates). We have at least two estimations of parturition dates for 91 ewes that were included in plasticity analyses. Mass, age and reproductive status were available for all these females. We adjusted female mass (kg) for capture date using linear mixed-effects models with restricted maximum likelihood where both the intercept and the slope were allowed to vary for each individual (Martin and Pelletier, 2011). Female mass adjusted to 15 September was treated as a continuous variable. 'Maternal mass in September' in analyses is this adjusted mass in September prior to parturition. We defined yearly reproductive success as a three-level factor variable : 'did not reproduce' (n = 82 female-years), 'did not wean a lamb' (n = 123) and 'weaned a lamb' (n = 194).

### 2.5.2 Environmental and demographic variables

Precipitation (total rainfall plus water equivalent of snowfall in mm per day) and mean daily temperature (°C) were obtained from the Environment Canada meteorological station at Nordegg, about 20 km west of Ram Mountain. A sliding window was used to determine the relevant period over which to calculate average temperature and precipitation, using 'slidingwin' function from the R library 'climwin' (Bailey and van de Pol, 2016). Most conceptions are in late November–early December (Hogg, 1984 ; Hogg et al., 2017). As bighorn sheep show very little flexibility in gestation length (Hogg et al., 2017), we assumed that conception occurred 172 days before parturition. Therefore, we considered all possible windows of all possible lengths between the earliest parturition date (16 May) and the earliest predicted conception date (26 November). We used windows based on the same starting and ending dates for all individuals (an 'absolute' sliding window ; Bailey and van de Pol (2016)). We selected the window that provided the lowest AIC for models of log-transformed parturition date over the weather and climate variables being tested. Precipitation was thus averaged from 21 October to 15 November (AIC lower than model without precipitation by 22.16) while mean daily temperatures were averaged from 30 August to 19 November (AIC lower than model without temperature by 21.52) in the conception year. Total precipitation and mean daily temperatures are referred to as autumn precipitation and autumn temperature. Pacific Decadal Oscillation (PDO) values were available each year from 1992 (http://jisao.washington.edu/pdo). The PDO, similarly to the North Atlantic Oscillation, is a global climate index characterized by a 10-year cycle of

alternating cold and warm phases in Western North America. It is measured as the leading principal component of North Pacific monthly sea surface temperature variability (Mantua et al., 1997). Monthly values were averaged over the months of July and August prior to parturition ('summer PDO') based on a sliding window (AIC lower than model without PDO by 1.12). Population size (referred to as density) is quantified each year in June as the number of females aged 2 years and older (Pigeon et al., 2017).We used density in June in the year of conception to analyse parturition date. Density mostly declined after 1992 (Fig. 2.2a).

### 2.5.3 Temporal trends and determinants of parturition date at the population level

We used general linear models with a Gaussian error distribution to estimate annual trends in median parturition date (r = 0.90 between median and mean parturition dates), with year, autumn precipitation, autumn temperature, density and summer PDO fitted as continuous variables over the 26 years of study. To avoid spurious correlations, we removed the temporal effects from summer PDO, autumn precipitation, autumn temperature and density. Density and summer PDO were expressed as the residuals of a linear regression over year as a quadratic variable, while autumn temperatures and precipitation were expressed as the residuals of a linear regression over year. These variables are hereafter referred to as 'detrended' (Grosbois et al., 2008). We used linear mixed-effects models to test the effect of detrended precipitation, temperature and PDO as linear and quadratic terms on log-transformed parturition date, and their two-way interactions with detrended density. We conducted separate analyses for each predictor to avoid model over-fitting. Final models for each set of variables were determined by sequentially removing the least significant term from the model based on its *P* value. Significance of model terms was then assessed with likelihood ratio tests (LRTs; difference in log-likelihood between hierarchical models, tested against a chi-square distribution with the number of degrees of freedom that corresponded to the difference in the number of terms estimated). Our baseline model included maternal mass in September and previous reproductive success as fixed effects (Feder et al., 2008). All other models added one variable to the baseline model. Year and female identity were included as random effects in analyses of the effects of environmental determinants on log-transformed parturition date to account for pseudo-replication and unexplained annual variation in parturition date.

We then calculated repeatability from the baseline model, with and without year as a random effect, to measure how much of between- or within-individual differences could be attributed to year-to-year variation in parturition date. Repeatability is the amount of phenotypic variation that can be attributed to between-individual variation over the sum of between- and within-individual variations (Nakagawa and Schielzeth, 2013). The non-repeatable fraction of phenotypic variation should thus correspond to the sum of measurement error and individual phenotypic plasticity. In the absence of significant between-individual variation in slopes ($I \times E$), the variance attributable to individual identity will remain constant across all values of an environmental covariate and can be used to estimate the repeatability of a trait. We provide estimates and 95% confidence intervals calculated by parametric bootstrapping in R package 'rptR' (Nakagawa and Schielzeth, 2013).

### 2.5.4 Phenotypic plasticity

To quantify within-individual plastic responses of parturition date to environmental variation, we used random regression models. All environmental variables were standardized to zero mean and unit variance (see values prior to standardisation, Table A.1 in Supplementary Online Material). Given the lack of quadratic effects of autumn precipitation, autumn temperature, summer PDO and density at the population level, only their linear effects were tested at the individual level. To facilitate interpretation, we repeated plasticity analyses with both raw and detrended predictors. Estimates from the models fitted on detrended predictors are in the Supplementary Online Material. Maternal mass in September and previous reproductive success were included in our baseline model. Within-individual plastic responses can also be quantified using within-subject mean centering (van de Pol and Wright, 2009). Within-subject centering decomposes environmental effects into those associated with the average environment experienced by each individual over its lifetime (a 'between-individual' effect) and those attributable to yearly deviations of the environment from each individual's lifetime average (the 'within-subject' effect). Within-subject centering considers that individuals experience different ranges of environmental conditions over their lifetime and centering around subjects' means eliminates any between-subject variation. Each environmental covariate was thus subdivided into a within-individual ($\beta_W$) and a between-individual ($\beta_B$) component, by subtracting the individual's mean value ($\beta_B$, reflecting the population trend) from each individual observation ($x_{ij} - \overline{x}_j$, or $\beta_W$, reflecting plasticity). Following (van de Pol and Wright,

2009), we modelled parturition date ($y_{ij}$) for each individual $i$ in year $j$ as a continuous response to variation in each of the weather, climate or density variables. For example, the model for precipitation was as follows :

$$y_{ij} = \beta_0 + \beta_B \overline{Prec}_i + \beta_W (Prec_{ij} - \overline{Prec}_j) + mass + prs + [year] + [ID_i] + [ID_i (Prec_{ij} - \overline{Prec}_j)] + \varepsilon_{0ij}$$

where $\beta_0$ represents the intercept and, as the predictors (here precipitation) were standardized to zero mean, is equivalent to the expected parturition date in the average environment. The between-subject effect ($\beta_B$) is estimated as the slope of $y_{ij}$ on the mean value of each predictor for each individual, $i$. The within-subject ($\beta_W$) effect is estimated as the slope of $y_{ij}$ on the annual deviation of the predictor from the subject mean. 'Mass' and 'prs' refer to maternal mass the previous autumn and previous reproductive success, while $\varepsilon_{0ij}$ represents the residual error.

The presence of within-individual plasticity in the population could thus be investigated using within-subject mean centering, while variation in within-individual plasticity ($I \times E$) was investigated using random regression models (Nussey et al., 2007). The random effects are shown in square brackets in the previous equation : $[ID_i]$ quantifies the variation in within-subject intercepts, and $[ID_i(Prec_{ij} - \overline{Prec}_j)]$ quantifies the variation in the within-subject slopes for the effects of precipitation (Nussey et al., 2007). Year was included as a random effect to model variation across years that were not explained by environmental variables. We ran similar models for autumn temperature, summer PDO and female density. LRTs revealed that the random slope effects of the environmental variables were not significant (Table 2.1). We thus kept the random intercepts of identity and year in further analyses and in visual representations of individual plastic responses to precipitation and temperature.We included females with more than one parturition date to quantify their response to environment and to improve uncertainty estimates around within-individual slopes (n = 350 observations of 91 females out of 442 total observations, average of 3.85 parturition dates per female). All linear mixed models were computed using R library 'lme4' and function 'lmer' (Bates et al., 2015). We calculated a pseudo-$R^2$ marginal for fixed effects and a pseudo-$R^2$conditional combining fixed and random effects according to Nakagawa and Schielzeth (2013). We performed all analysis on R version 3.5.1 (R Core Team, 2018).

## 2.6 Results

### 2.6.1 Determinants of parturition date at the population level

**Table 2.1 Random regression analyses to assess variation in plasticity, i.e. an individual-by-environment ($I \times E$) component, in bighorn sheep parturition date (n = 350), 1992 to 2017, Ram Mountain, Alberta, Canada.**

| Precipitation | Log-likelihood | Test | df | LRT | P-value |
|---|---|---|---|---|---|
| 1. Year | -285.33 | - | 8.00 | - | - |
| 2. Year + Female | -281.91 | 1 vs 2 | 9.00 | 6.86 | 0.009 |
| 3. Year + Female x Precipitation$_{within}$ | -281.22 | 2 vs 3 | 11.00 | 1.37 | 0.50 |
| Temperature | | | | | |
| 4. Year | -287.42 | - | 8.00 | - | - |
| 5. Year + Female | -284.74 | 4 vs 5 | 9.00 | 5.36 | 0.02 |
| 6. Year + Female x Temperature$_{within}$ | -283.40 | 5 vs 6 | 11.00 | 2.67 | 0.26 |
| Density | | | | | |
| 7. Year | -293.32 | - | 8.00 | - | - |
| 8. Year + Female | -289.62 | 7 vs 8 | 9.00 | 7.40 | 0.007 |
| 9. Year + Female x Density$_{within}$ | -289.59 | 8 vs 9 | 11.00 | 0.07 | 0.97 |
| Summer PDO | | | | | |
| 10. Year | -296.46 | - | 8.00 | - | - |
| 11. Year + Female | -293.03 | 10 vs 11 | 9.00 | 6.86 | 0.009 |
| 12. Year + Female x PDO$_{within}$ | -291.62 | 11 vs 12 | 11.00 | 2.83 | 0.24 |

Three models with an increasingly complex random structure were compared for each of the four environmental variables : autumn precipitation, temperature, adult female density and summer PDO. Random structures of models 1 to 3, 4 to 6, 7 to 9 and 10 to 12 were compared with a LTR. All models included the within-individual ($\beta_W$) and the between-individual ($\beta_B$) components of the environmental variable as fixed predictors and maternal mass in September and previous reproductive success as control variables. Within-individual centering was applied as suggested by van de Pol and Wright (2009). Results from random regressions on 'detrended' predictors are found in Table A.3, and estimates of models with $I \times E$ are presented in Table A.5 of the Supplementary Online Material

Most births in 1992–2017 occurred in late spring (median date : May 30, mean date $\pm$ SD : June 3 $\pm$ 12.4 days), with 80% of lambs born in the 27 days after May 16$^{th}$ (Fig. 2.1). Median parturition date advanced by approximately 15.7 days over 26 years ($\beta$ = - 0.63 [- 0.98 to - 0.27], adj-$R^2$ = 0.34; Fig. 2.2b). During this time, the number of adult females decreased from 103 to 37 ($\beta_{Year}$ = - 1231 [- 1466.54 to - 995.14], $\beta^2_{Year}$ = 0.31 [0.25-0.37], adj-$R^2$= 0.94; Fig. 2.2a). Density declined from 1992 to 2003, then stabilized and slightly increased, partly

because of translocation of new individuals in 2003–2015 (Poirier and Festa-Bianchet, 2018). Daily precipitation decreased by 0.7 mm over the study period ($\beta_{Year}$ = - 0.03 [- 0.06 to - 0.003], adj-$R^2$ = 0.16; Fig. 2.2c) and autumn temperature increased by 2.9 °C ($\beta_{Year}$ = 0.12 [0.07- 0.16], adj-$R^2$ = 0.54) (Fig. 2.2d). There was no clear trend in summer PDO ($\beta_{Year}$ = - 28.32 [- 67.69 to 11.06], $\beta^2_{Year}$ = 0.01 [0.00-0.02], adj-$R^2$ = 0.08; Fig. 2.2e).

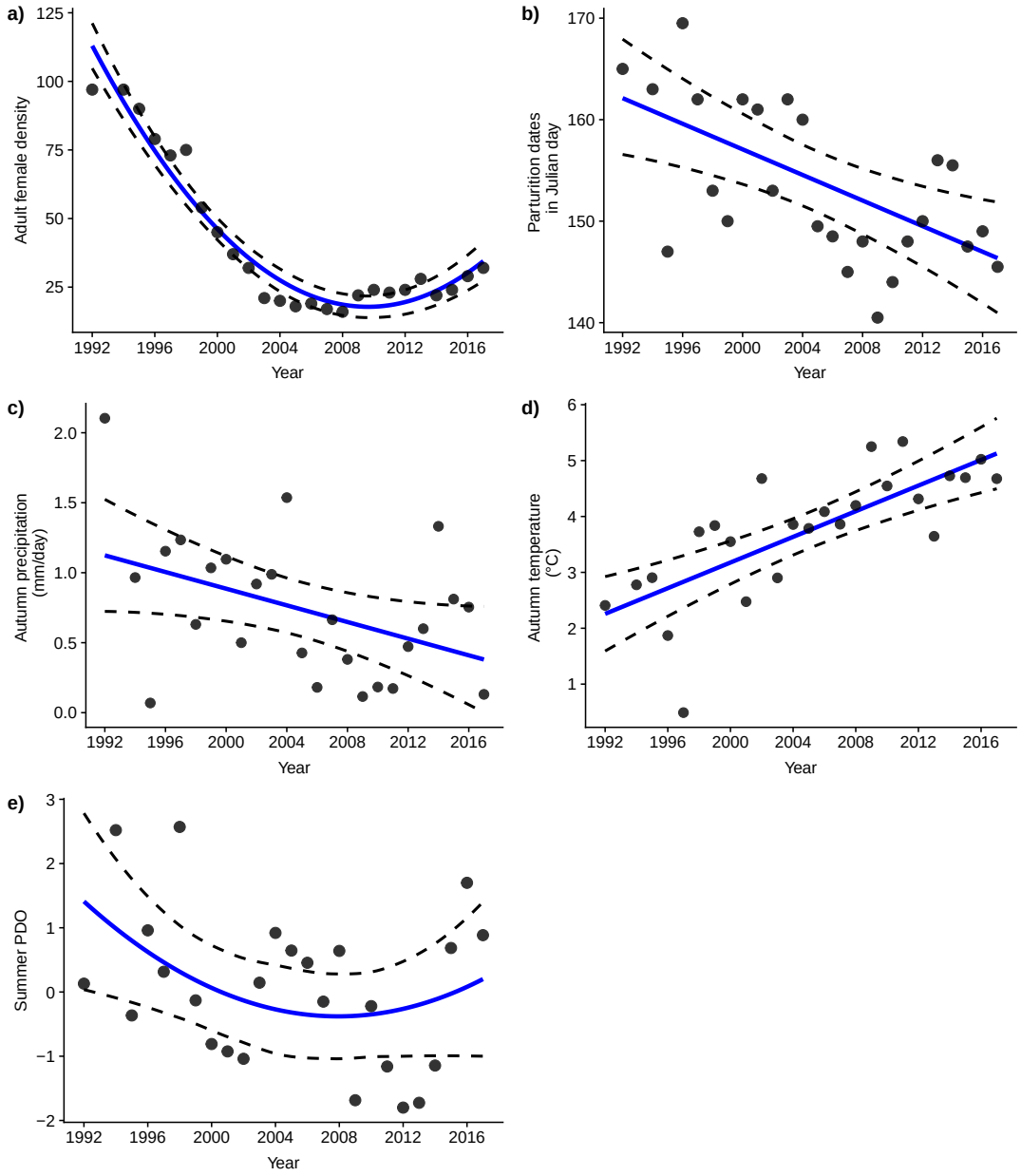


**Figure 2.2 Temporal trends at the population level in a adult female density, b median parturition date, c autumn precipitation (mm/day), d autumn temperature (°C) and e summer PDO at Ram Mountain, Alberta, Canada, 1992 to 2017.**

All predictors were measured during the year of conception, previous to parturition, up to 2016. Grey circles represent annual values and lines are model predictions (dotted lines : 95% CI)

At the population level, all results include parameter estimates for detrended environmental and density variables. The final models for precipitation and temperature only included linear effects of precipitation and temperature (Fig. 2.3a, b ; Table A.2). Increasing precipitation was associated with a delay in parturition of $\sim$17.3 days over the study period (Fig. 2.3a ; Table A.2), but the effect of temperature was not significant. There was no significant effect of density, either as a linear or quadratic term or in interaction with climate or weather variables, on log-transformed parturition date. Effects of summer PDO were also negligible (Table A.2). Reproductive success was treated as a three-level factor ; relative to females that had not reproduced the previous year ($\beta$ = 2.77 [2.59-2.97]), females that weaned a lamb delayed parturition by $\sim$ 1.3 days ($\beta$ = 2.85 [2.69-3.00]) and those that lost their lamb during summer advanced parturition by $\sim$ 2.0 days ($\beta$ = 2.63 [2.47-2.79] ; Fig. 2.4a). Heavier females gave birth slightly earlier : mass in September advanced parturition by $\sim$ 7.6 days over the observed range of standardized maternal mass ($\beta$ = - 0.10 [- 0.16 to - 0.03] ; Table A.2 ; Fig. 2.4b).

After accounting for maternal mass in September and previous reproductive success, repeatability was weak but significant (R = 0.21 [0.10–0.32]) suggesting that $\sim$ 21% of variation in parturition date was attributed to individual differences. When year was included as a random effect, repeatability was reduced to 0.07 [0.01–0.15], suggesting that part of the between-individual differences in parturition date can be explained by year-to-year variations in the environment. Adding year decreased the residual variance ($\text{Var}_{residual}$ = 0.25 [0.20– 0.29]), but whether or not year was included in the model, the ratio of residual variance to between-individual variance was high, suggesting a potential for phenotypic plasticity in response to changes in the environment.

### 2.6.2 Phenotypic plasticity in parturition date

Plasticity analyses included only linear effects of environmental variables. We present model estimates for random regressions and plasticity analyses fitted on raw predictors in the main text and in Tables 2.1 and 2.2. The equivalent models fitted on detrended predictors can be found in the Supplementary Online Material in Tables A.3 and A.4. We found significant individual plasticity in response to autumn precipitation ($\beta_W$ = 0.28 [0.16–0.40]) and to autumn temperature ($\beta_W$ = - 0.17 [- 0.32 to - 0.01]), but not to adult female density ($\beta_W$ = 0.04 [- 0.18 to 0.26] and summer PDO ($\beta_W$= 0.09 [- 0.08 to 0.26] ; Table 2). Random regression ana-

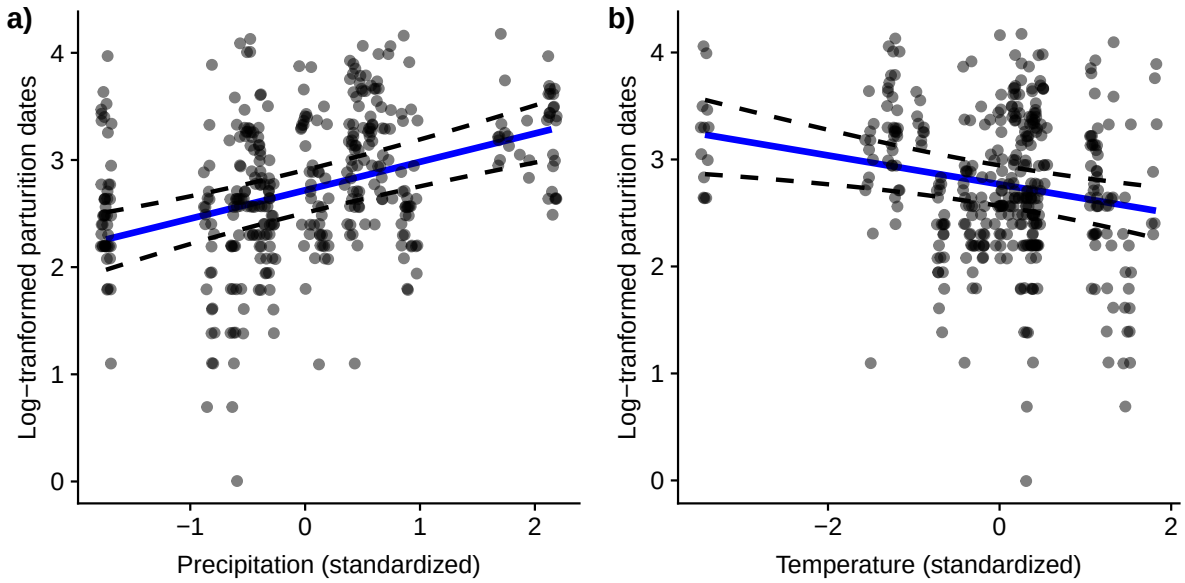


**Figure 2.3 Determinants of parturition date at the population level for bighorn sheep at Ram Mountain, Alberta, Canada, 1992 to 2017.**

Effects of **a** mean daily autumn precipitation ('detrended') and **b** mean autumn temperature ('detrended') on parturition date are illustrated. Parturition date was log-transformed starting at 0 on Julian day 136, the earliest recorded parturition. Previous reproductive success and maternal mass in September were added as fixed effects (see Fig. 2.4), and female identity and year as random intercepts. Transparent, grey circles represent annual raw values. The blue line is a model prediction for a female of average mass that did not produce a lamb the previous year (dotted lines : 95% CI)

lyses showed no variability of individual slopes of the relationships between environmental predictors and parturition date (no $I \times E$ for precipitation, temperature, density or PDO) in the random part of models 3, 6, 9 and 12 in Table 2.1 (but see full model estimates with $I \times E$ in Table A.5). Variance in intercepts for female identity was 0.03 in all models (Table 2.2, random effects). Estimates for between-individual slopes showed effects for autumn precipitation ($\beta_B$ = 0.43 [0.26-0.60]) and temperature ($\beta_B$ = - 0.34 [- 0.49 to - 0.20]; Fig. 2.5) and average density ($\beta_B$ = 0.21 [0.05-0.37]; Table 2.2). Effect of summer PDO ($\beta_B$ = 0.09 [- 0.11 to 0.29]) was not significant. Results were similar when detrended predictors were used, except that within-individual plasticity in response to temperature was not significant (Tables A.3 and A.4).

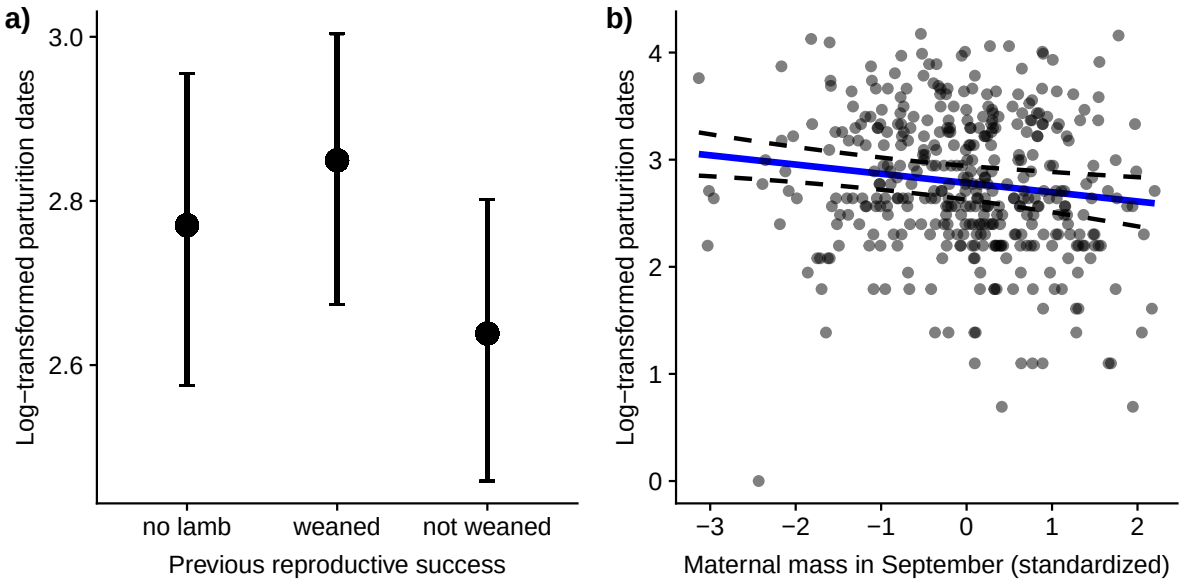


**Figure 2.4 Effects of a previous reproductive success and b maternal mass in September on parturition date at the population level for bighorn sheep at Ram Mountain, Alberta, Canada, 1992 to 2017.**

Estimates are for a female of average mass in **a**. No lamb : no lamb produced the previous year ; weaned : weaned a lamb the previous September ; not weaned : lamb produced the previous year died over the summer, including neonatal mortality. In **a**, transparent, grey circles represent annual raw values and the blue line is a model prediction for a female that did not produce a lamb the previous year (dotted lines : 95% CI). All estimates are from model 2 in Table 2.1

In addition, we investigated whether heterogenous sampling of individuals over the study period, with some females monitored only at low or high density, led to a spurious relationship of adult female density with log-transformed parturition date. Based on median density over the study period (median = 32, standardized value = - 0.53), we separated years in two subgroups, low and high density, and repeated plasticity analyses for each subgroup. The between-individual density effect on parturition date was no longer significant ($\beta_B$(low)= 0.03 [- 1.88 to 1.92], $\beta_B$(high)= 0.17 [- 0.06 to 0.40] ; Table A.6), based on the model without $I \times E$ in both subgroups.

Finally, if within-individual plasticity explains between individual responses, their estimated slopes should be similar and $\beta_B - \beta_W$ should be near zero and non-significant. We thus as-

sessed whether within- and between-individual slopes were different by rewriting our initial model (e.g. precipitation) as follows :

$$y_{ij} = \beta_0 + (\beta_B - \beta_W)\overline{Prec_i} + \beta_W(Prec_{ij}) + mass + prs + [year] + [ID_i] + [ID_i(Prec_{ij} - \overline{Prec_j})] + \varepsilon_{0ij}$$

where the original fixed predictor effect $Prec_{ij}$ now combines both within- and between-individual effects, and $\overline{Prec_i}$ only expresses between-individual variation while controlling for within-individual effects. These analyses suggested a weak and non-significant difference between slopes for precipitation ($\beta_B - \beta_W$ = 0.15 [- 0.02 to 0.32]), but the difference in slopes for temperature was significant ($\beta_B - \beta_W$ = - 0.18 [- 0.34 to - 0.01], see equation 3 in van de Pol and Wright 2009 for more details on the method ; Table A.7). Detrended predictors gave slightly different results : the difference in slopes was not significant for precipitation ($\beta_B - \beta_W$ = 0.03 [- 0.14 to 0.20]) but the difference in slopes for temperature was marginal ($\beta_B - \beta_W$ = - 0.08 [- 0.23 to 0.08]) and excluded the presence of plasticity in response to detrended temperature ($\beta_W$ = - 0.12 [- 0.27 to 0.03] ; Table A.4). Sample size influenced our capacity to detect plasticity. As the number of observations/female increased to 7 ($n_{females}$ = 13), between- and within-individual slope estimates increased in the model including adult female density ($\beta_B$ = 0.84 [- 0.96 to 2.60]), $\beta_W$ = 0.68 [- 0.17 to 1.58]) ; Fig. A.1) but large confidence intervals and a large difference in slopes ($\beta_B - \beta_W$ = 0.16 [- 1.58 to 1.89]) suggest that plasticity alone cannot account for all the observed population change. Increasing number of observations/female also increased the magnitude of the within-individual precipitation effect. In all models, however, sample size greatly decreased as number of observations/female increased from 2 ($n_{females}$ = 91) to 7 ($n_{females}$ = 13), resulting in wider confidence intervals (Fig. A.1).

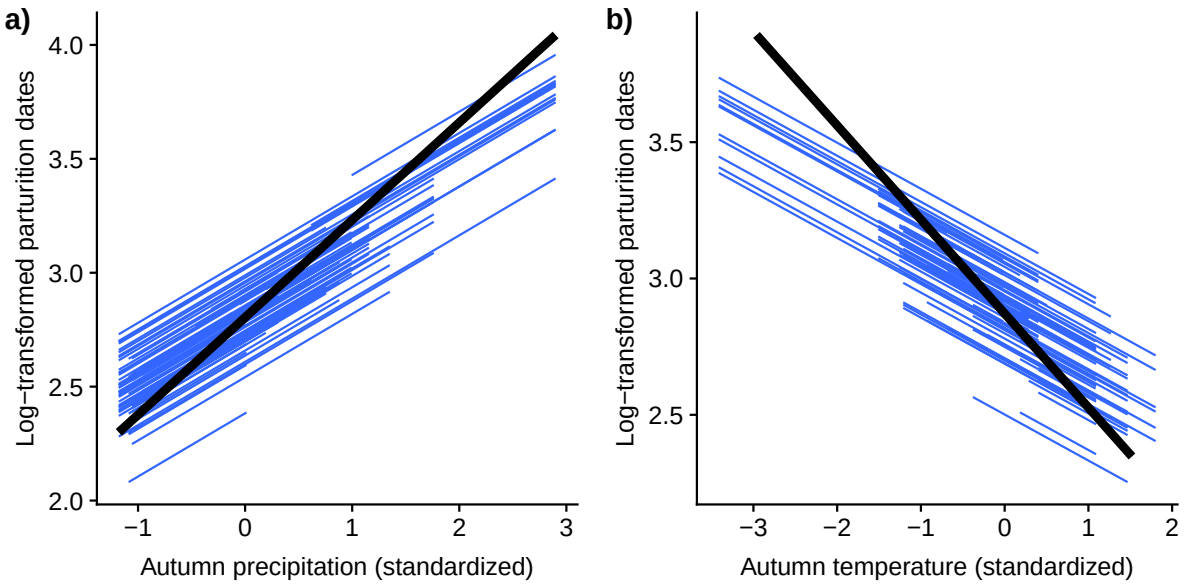


**Figure 2.5 Effects of a mean daily autumn precipitation and b mean autumn temperature on parturition date for bighorn sheep at Ram Mountain, Alberta, Canada, 1992 to 2017.**

Parturition date was log-transformed starting at 0 on Julian day 136, the earliest recorded parturition. Previous reproductive success and maternal mass in September were added as fixed effects. Female identity and year were added as random intercepts (Table 2.1). Within-individual centering ($\beta_B$ vs $\beta_W$) was applied as suggested by van de Pol and Wright (2009), and initial predictors (not 'detrended') were included in models. Thick, black lines represent the between-individual trend ($\beta_B$), and thin, blue lines represent within individual reaction norms ($\beta_W$) obtained from predictions of linear mixed effects models for a female of average mass that did not produce a lamb the previous year

**Table 2.2 Parameter estimates of linear mixed-effects models of the effects of between-individual ($\beta_B$) and within-individual ($\beta_W$) components of (a) precipitation, (b) temperature, (c) adult female density and (d) summer PDO on 350 bighorn sheep parturition date, 1992 to 2017, Ram Mountain, Alberta, Canada.**

| Fixed effects | Estimate | 95% CI | Random effects | Variance |
|---|---|---|---|---|
| a) Autumn precipitation | | | | |
| Intercept (no lamb) ($\beta_0$) | 2.80 | 2.62 - 2.99 | Female (Intercept) | 0.03 |
| Maternal mass | -0.09 | -0.16 - -0.02 | Year (Intercept) | 0.06 |
| PRS : not weaned | -0.18 | -0.37 - 0.01 | Residual | 0.25 |
| PRS : weaned | 0.03 | -0.14 - 0.22 | $R^2$marginal | 0.26 |
| Precipitation$_{between}$ ($\beta_B$) | 0.43 | 0.26 - 0.60 | $R^2$conditional | 0.46 |
| Precipitation$_{within}$ ($\beta_W$) | 0.28 | 0.16 - 0.40 | | |
| b) Autumn temperature | | | | |
| Intercept (no lamb) ($\beta_0$) | 2.87 | 2.67 - 3.07 | Female (Intercept) | 0.03 |
| Maternal mass | -0.10 | -0.17 - -0.03 | Year (Intercept) | 0.09 |
| PRS : not weaned | -0.21 | -0.39 - -0.01 | Residual | 0.25 |
| PRS : weaned | 0.01 | -0.17 - 0.19 | $R^2$marginal | 0.22 |
| Temperature$_{between}$ ($\beta_B$) | -0.34 | -0.49 - -0.20 | $R^2$conditional | 0.46 |
| Temperature$_{within}$ ($\beta_W$) | -0.17 | -0.32 - -0.01 | | |
| c) Female density | | | | |
| Intercept (no lamb) ($\beta_0$) | 2.87 | 2.66 - 3.09 | Female (Intercept) | 0.03 |
| Maternal mass | -0.09 | -0.17 - -0.03 | Year (Intercept) | 0.13 |
| PRS : not weaned | -0.20 | -0.39 - -0.01 | Residual | 0.24 |
| PRS : weaned | 0.02 | -0.16 - 0.20 | $R^2$marginal | 0.11 |
| Density$_{between}$($\beta_B$) | 0.21 | 0.05 - 0.37 | $R^2$conditional | 0.46 |
| Density$_{within}$ ($\beta_W$) | 0.04 | -0.18 - 0.26 | | |
| d) Summer PDO | | | | |
| Intercept (no lamb) ($\beta_0$) | 2.85 | 2.62 -3.07 | Female (Intercept) | 0.03 |
| Maternal mass | -0.09 | -0.16 - -0.02 | Year (Intercept) | 0.16 |
| PRS : not weaned | -0.19 | -0.37 - 0.01 | Residual | 0.25 |
| PRS : weaned | 0.04 | -0.14 - 0.22 | $R^2$marginal | 0.05 |
| PDO$_{between}$($\beta_B$) | 0.09 | -0.11 - 0.29 | $R^2$conditional | 0.47 |
| PDO$_{within}$($\beta_W$) | 0.09 | -0.08 - 0.26 | | |

Estimates of fixed effects and variance components of random effects of models 2, 5, 8 and 11 are presented based on results in Table 1. Within-individual centering was applied as suggested by van de Pol and Wright (2009). PRS : reproductive success the previous year. Model estimates from 'detrended' predictors can be found in Table S4 and estimates of models with $I \times E$ are presented in Table S5 of the Supplementary Online Material

## 2.7 Discussion

Mean parturition date in the study population now occurs 15 days earlier than 26 years ago. Our investigation of the factors contributing to this change showed the importance of autumn precipitation and, to a lesser extent, autumn temperature. Our study area is experiencing a long-term increase in temperature and a decrease in precipitation (Douhard et al. (2017) ; this study), underpinning the need for bighorn sheep to track phenological changes. Individuals responded plastically to autumn precipitation, but not to autumn temperature, population density or summer PDO.We did not find evidence of individual differences in female capacity to adjust parturition date in response to environmental changes, as suggested by the absence of significant individual-by-precipitation or individual-by-temperature variability in our analyses. Our results suggest a rapid population response to changes in precipitation, likely due to a plastic response at the individual level rather than to evolutionary processes.

### 2.7.1 Long-term changes in phenology

Female bighorn sheep advanced parturition date by about 0.63 day/year over the study period. This rate is higher than the 0.42 day/year $\pm$ 0.08 SE) advance in parturition date or the 0.26 day/year $\pm$ 0.07 SE) advance in oestrus date reported in red deer in response to warming temperatures (Moyes et al., 2011 ; Stopher et al., 2014). Our analyses of the determinants of parturition date at the population level suggest that local weather, rather than large-scale climate indices or density, is more important for bighorn sheep reproductive phenology. We used a sliding window to determine the relevant period over which to quantify the effects of weather and climate variables on parturition date in the population. This window varied from July (PDO) to November (temperature and precipitation), thus covering late summer and autumn. Dry and warm autumns were associated with earlier parturition, suggesting earlier conception. Gestation length in bighorn sheep shows very little flexibility ; therefore, conception date should be closely correlated with parturition date (Hogg et al., 2017), in contrast to other ungulates that show flexibility in gestation length (Clements et al., 2011). We thus hypothesize that mild autumns indirectly affect female body mass before conception through improved foraging conditions.

Changes in parturition date could synchronize vital activities—such as lactation and subsequent conception—with changes in food abundance. Hogg et al. (2017) showed, in another bighorn sheep population, that females conceived earlier when summer and winter evapotranspiration, an index of food resources, increased. Seasonal precipitation improved body condition and advanced bighorn sheep parturition in their study area in Montana, at lower elevation and with a much drier climate than Ram Mountain. Mild autumn conditions could also favour earlier weaning of lambs, as reported in feral sheep (Forchhammer et al., 2001), and trigger early return to oestrus. Because most precipitation in late autumn falls as snow in our study area, and deep snow may decrease forage availability, one may expect high autumn precipitation to increase female energy expenditure and lower body condition, thus potentially delaying conception date (Feder et al., 2008).

Regardless of the mechanisms triggering conception in ewes and whether large-scale climate indices or local weather variables are measured, the association between parturition dates and their determinants suggests that bighorn sheep can partly track phenological changes. Our results also highlight the importance of cues other than temperature (Thackeray et al., 2010; Siepielski et al., 2017) as drivers of reproductive phenology in ungulates. For example, latitude explains variation in birth season length (the number of days in which 80% of all births occur) in captive ruminants, supporting the hypothesis that photoperiod—although independent of climate change—has an important impact on ruminant reproduction (Zerbe et al., 2012).

We expected that increasing density would delay reproduction because of density-dependent competition for resources. Contrary to our prediction, there was no significant effect of density on parturition date. However, density effects remain difficult to assess due to potential time lags in population response. Finally, important within-individual variability led to low repeatability estimates of parturition date. Repeatability estimates of parturition date in bighorn sheep were lower than those measured in five populations of roe deer (range R = 0.54–0.93), which are the highest reported for any mammal, suggesting low plasticity in this trait in that species (Plard et al., 2012). We thus suggest that intra-individual responses were expressed as phenotypic plasticity in response to environmental changes in bighorn sheep.

### 2.7.2 Plasticity analyses of parturition date

Using linear mixed-effects models and mean-centering, we detected plasticity in parturition date in response to autumn precipitation. The difference in between- and within individual slope estimates ($\beta_B - \beta_W$) was near zero, suggesting that the population and individual responses are effectively the same and that individual plasticity (within-individual component, $\beta_W$) may drive a population response to autumn precipitation. Autumn precipitation may be a cue for timing of reproduction in bighorn sheep. Whether this cue is related to changes in plant phenology or to other mechanisms is unknown, but patterns observed at the population and individual levels were very similar in direction and magnitude, strongly suggesting that ewes adjust parturition date—through conception date—to changing environmental conditions. With climate change, earlier breeding may improve the match between parturition and optimal environmental conditions. Whether or not the phenotypic responses observed in this study are beneficial over the long term, however, will require further investigations of the consequences of plasticity on fitness in this population.

There was no plastic response to summer PDO or density, and the temperature effect disappeared when testing detrended temperature. The absence of effects, either at the population or individual level, does not seem to be entirely caused by an insufficient sample size. Increasing number of measurements per female increased the between-individual components of density, temperature and precipitation, but only the within-individual precipitation effect. Between- and within-individual density effects were not significant and had large confidence intervals resulting from small sample size. Summer PDO effects were independent of the number of observations/ female. We finally investigated whether the between-individual density effect could be due to a heterogenous sampling of individuals. Between- or within-individual density effects on parturition dates were not significant when analyses were restricted to either high or low density, or when density was detrended. Large differences in within- and between-individual slope estimates also suggest that plasticity did not explain the between-individual density effect when all females were included in analyses of 'raw' predictors. Altogether, these results suggest that a temporal trend in density or heterogenous sampling of individuals explained a significant between-individual density effect and that plasticity could not explain this apparent response to density. Mechanisms for density effects in our study population thus remain unknown.

Finally, it is possible that other, unmeasured ecological variables drive plasticity in parturition date in bighorn sheep. However, our analyses are very conservative since we modelled year-to-year variations in parturition date that were not explained by environmental covariates, both at the population and individual levels, by including year as a random effect. Including year also accounted for multiple measures per year, which might lead to non- independent observations within years. Modelling this year-to-year variation possibly decreases the annual variability explained by covariates of interests such as precipitation, temperature, climate or density ; however, the relatively high conditional coefficients of determination ($R_c^2 = 46 - 47\%$) suggest that our models effectively explained variation in parturition date while also excluding potential confounding temporal trends in covariates. Rapid climate change occurs worldwide, and phenotypic plasticity may be key to population persistence. Shifting climates, particularly changes in precipitation patterns, present a challenge for many organisms (Siepielski et al., 2017). Importantly, local and regional climate change may explain patterns of phenology much better than global change (Siepielski et al., 2017). For example, in our study, local precipitation was an important variable affecting parturition date. We found population- and individual-level changes, suggesting an effect of weather on the reproductive phenology of bighorn sheep. Variation in plasticity was very weak or could not be detected in response to changes in precipitation, temperature, climate and adult female density. Parturition date was mostly driven by autumn weather, suggesting that reproductive phenology in large mammals may be more dependent on variation in the timing of ovulation rather than in spring or summer resource acquisition.

### 2.7.3 Acknowledgements

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## 2.8 Compliance with ethical standards

### 2.8.1 Ethical standards

Animals were captured and handled in compliance with the Canadian Council on Animal Care, under the approval of the Université de Sherbrooke Animal Care Committee (protocol number FP- 2016-01 and MFB-2014-01—Université de Sherbrooke).

### 2.8.2 Conflit of interest

The authors declare that they have no conflict of interest.

### 2.8.3 Data availability

The datasets analyzed during the current study are available from the corresponding author on reasonable request.

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CHAPITRE 3

# LES CAUSES ET CONSÉQUENCES À COURT TERME DE LA VARIATION EN COMPOSITION DU LAIT

*In nature nothing exists alone.* Rachel Carson, Silent Spring

## 3.1 Description de l'article et contribution

L'écophysiologie est aux prises avec une problématique de taille, celle de la multitude et la complexité des marqueurs physiologiques disponibles. Ces marqueurs peuvent se révéler peu concluants lorsque mis en lien avec des paramètres biologiques de valeur adaptative, tels le succès reproducteur et la survie. Dans cet article, nous appliquons aux données de la composition du lait de brebis une approche initialement appliquée à l'écologie des communautés, le modèle hiérarchique multivarié avec variables latentes. La méthode facilite la décomposition des matrices de (co)variances typiques des traits physiologiques, comportementaux ou morphologiques étudiés en milieu naturel. L'objectif général de l'article était de tester l'hypothèse selon laquelle la composition du lait peut avoir des conséquences sur le succès reproducteur à court terme. Les objectifs spécifiques étaient 1) d'évaluer la présence de différences répé s en composition du lait entre les brebis, 2) de déterminer l'importance relative des différences interannuelles et interindividuelles en composition du lait, et 3) de lier la physiologie aux traits d'histoire de vie importants pour la valeur adaptative pour finalement, 4) de vérifier si la composition du lait est corrélée aux changements en masse des femelles et à la survie au sevrage des agneaux.

Pour cet article, j'ai élaboré et effectué les analyses avec Guillaume Blanchet et Alan Cohen. J'ai ensuite écrit de la première version du manuscrit. Fanie Pelletier, Guillaume Blanchet et Alan Cohen ont par la suite commenté plusieurs versions du manuscrit, et ont contribué à l'interprétation des données et à la révision du manuscrit. J'ai participé aux travaux de terrain ainsi qu'à l'extraction des composantes physiologiques en laboratoire.

**Causes and short-term consequences of variation in milk composition in wild sheep**

*Journal of Animal Ecology* (2019) 88 : 857–869.

Limoilou-Amelie Renaud, F. Guillaume Blanchet, Alan A. Cohen et Fanie Pelletier

## 3.2 Abstract

1. Ecologists seek to understand the fitness consequences of variation in physiological markers, under the hypothesis that physiological state is linked to variability in individual condition and life history.
2. Thus, ecologists are often interested in estimating correlations between entire suites of correlated traits, or biomarkers, but sample size limitations often do not allow us to do this properly when large numbers of traits or biomarkers are considered.
3. Latent variables are a powerful tool to overcome this complexity. Recent statistical advances have enabled a new class of multivariate models—multivariate hierarchical modelling (MHM) with latent variables—which allow to statistically estimate unstructured covariances/correlations among traits with reduced constraints on the number of degrees of freedom to account in the model. It is thus possible to highlight correlated structures in potentially very large numbers of traits.
4. Here, we apply MHM to evaluate the relative importance of individual differences and environmental effects on milk composition and identify the drivers of this variation. We ask whether variation in bighorn sheep milk affects offspring fitness.
5. We evaluate whether mothers show repeatable individual differences in the concentrations of 11 markers of milk composition, and we investigate the relative importance of annual variability, maternal identity and morphological traits in structuring milk composition. We then use variance estimates to investigate how a subset of repeatable milk markers influence lamb summer survival.
6. Repeatability of milk markers ranged from 0.05 to 0.64 after accounting for year-to-year variations. Milk composition was weakly but significantly associated with maternal mass in June and September, summer mass gain and winter mass loss. Variation explained by year-to-year fluctuations ranged from 0.07 to 0.91 suggesting a strong influence of environmental variability on milk composition. Milk composition did not affect lamb survival to weaning.

7. Using joint models in ecological, physiological or behavioural contexts has the major advantage of decomposing a (co)variance/correlation matrix while being estimated with fewer parameters than in a "traditional" mixed-effects model. The joint models presented here complement a growing list of tools to analyse correlations at different hierarchical levels separately and may thus represent a partial solution to the conundrum of physiological complexity.

**Keyword :** Bayesian modelling, ecophysiology, hierarchical levels, individual differences, lactation, maternal strategy, multivariate analyses

## 3.3 Introduction

One challenge in ecology and evolution is to quantify how processes acting at different spatiotemporal scales affect different levels of biological organization (Pigeon et al., 2013) and to evaluate how ecophysiological responses to the environment influence organismal fitness. Ecophysiology is the study of how the environment interacts with organismal physiology, and involves many physiological systems such as the immune response (Pigeon et al., 2013), oxidative stress (McGraw et al., 2010) and the stress response (Bonier et al., 2009a). Within each physiological system lie complex networks of interacting units (Cohen et al., 2012); the failure of any unit may be associated with deleterious consequences for the health of an individual, including increased risks of infections (Arsnoe et al., 2011) and decreased overall condition (Wikelski and Cooke, 2006). Individual differences in physiological condition can contribute to variance in reproductive success and ultimately individual fitness (Seebacher and Franklin, 2012).

Understanding how variability in ecophysiological systems—and all their interacting units—affects individual fitness is challenging (but see Buehler et al. (2011); Milot et al. (2014)). One difficulty is to obtain repeated measurements on wild individuals of known age and life history and to reliably estimate level-specific correlations among traits (i.e., within biological levels of organization, e.g., groups, years or individuals). Often measurements of several biomarkers (e.g., thyroid hormones, haematocrit, plasma volume, metabolic rate—Elliott et al. (2015)) are only available for a few individuals, thus leading to sample size limitations and large numbers of correlated traits. However, the greatest difficulty mostly comes from unstable correlations among markers, since physiological "snapshots" may not reflect physiological levels in previous life-history stages (Bonier et al., 2009b). Several lines of enquiry are thus often used to investigate links with reproductive success or fitness. Searching through all the biomarkers that distinguish healthy individuals from unhealthy ones is an option (Davis, 2008) Another theme is to extract a signal of homeostasis as a more holistic assessment of condition (Milot et al., 2014). Both options require multiple biomarkers, and disentangling sources and scales of variation in physiological, ecological or behavioural traits using integrative methods is essential to ultimately link ecophysiology to fitness and life-history strategies in ecological populations.

Level-specific correlations among traits can be modelled using latent variables, that is, some unobserved ('latent') predictors. In multivariate generalized mixed models (GLMM; Bolker

et al., 2009), correlations are estimated using a multivariate random effect (i.e., a latent variable ; Warton et al., 2015). The multivariate random effect typically is assumed to have a completely unstructured (co)variance matrix ; the number of parameters thus increases quickly as the number of traits increases. Latent variable models are also a function of unmeasured predictors ; random effects must be linearly related to a set of latent variables. Using factor analysis, a form of latent variable models (Thorson et al., 2015), genetic and environmental variances can be obtained through estimation of leading eigenvectors in quantitative genetics (Kirkpatrick and Meyer, 2004 ; Meyer, 2009). Similarly, Walling et al. (2014) tackle the problem of using bivariate correlations to study genetic constraints in life-history traits, by modelling multiple traits altogether. Bivariate correlations can underestimate the genetic (co)variances across traits and be poor indicators of genetic constraints. In community ecology, joint models of species distribution have been commonly specified using either GLMM or latent variable models (Warton et al., 2015), where information (i.e., correlations) from multiple species is considered simultaneously to study their responses to their environment both individually and at the community level (Ovaskainen et al., 2017). Recent advances in Bayesian modelling allow specification of appropriate priors, permitting uncertainty in the number of latent variables (or random effects) and sparsity structure (Bhattacharya and Dunson, 2011 ; Ovaskainen et al., 2017).

Here, we extend the use of Bayesian joint modelling to study the physiological processes that structure milk composition (Figure 3.1). Milk composition, such as fat content, reflects a direct and expensive transfer of energy from mother to offspring (Oftedal, 2000) ; it is thus a crucial aspect of early life conditions and is also likely to have long-term effects on the fitness of young individuals (Lindström, 1999). Two important life-history parameters, body mass and growth rate of young (Iverson et al., 1993 ; Mellish et al., 1999), have been shown to be influenced by milk composition, but several other life-history parameters can be affected by the nutritive, immunological and hormonal constituents in milk (e.g., immune, cognitive and neurobiological development in infants ; Hinde and Milligan (2011)). Focusing on its nutritional aspects, milk is often described by two well-known nutritive components—average fatty acid and protein concentrations—and total gross energy derived from these components, especially in wild mammals (e.g., Skibiel and Hood, 2015 ; Quesnel et al., 2017). Only a few studies, however, have investigated the simultaneous sources of variation—environmental and individual— on wild mammals' milk composition including mineral, fat, protein and sugar contents, despite their evolutionary importance (Cook and Baker (1969) ; Carlini et al. (1994) ; see review by

Skibiel et al. (2013) in captive and wild species).

Here, we apply multivariate hierarchical modelling (MHM) to analyse data on 11 markers of milk composition of bighorn ewes (*Ovis canadensis*)—fatty acids, proteins, sugars and minerals—to test the hypothesis that milk composition can have short-term fitness consequences. To do so, we (a) evaluate whether mothers show repeatable individual differences in milk composition and (b) investigate how annual variability influences differences between mothers in milk composition. We then investigate whether females allocate fewer resources to lactation by linking important life-history parameters to their physiology. June and September female body mass, female winter mass loss and summer mass gain are important fitness-related traits in bighorn sheep (Douhard et al., 2018 ; Pelletier et al., 2007). As body mass is correlated with condition in ungulates (Festa-Bianchet, 1998 ; Parker et al., 1993), it is likely to affect milk composition. MHM revealed that for three milk markers more than 10% of variation was explained by mother's identity, suggesting that the expected links between milk composition and maternal characteristics are explained by a small subset of markers. We thus asked whether (c) milk composition is correlated with female June and September mass and seasonal mass changes, and whether (d) milk composition affects lamb survival to weaning.

The major advantage of using joint modelling (i.e., MHM) in an ecophysiology context is the decomposition of a (co)variance/correlation matrix using a reduced number of latent variables, which allows approximation of the "full" matrix using fewer parameters than in a "standard" mixed-effects model. Most ecophysiology studies have small sample sizes relative to the number of biomarkers measured, and few degrees of freedom, and may thus require methods such as this one. Globally, the model structure is similar to factor analytic mixed models used to analyse multivariate phenotypes of individuals (Walling et al., 2014). In an ecophysiology context, MHM also extracts a maximum of information from the data while using a minimum number of parameters—and degrees of freedom—to estimate the model, similar to using only a few principal components axes to quantify the major fraction of variation in a dataset (Buehler et al., 2011 ; Pigeon et al., 2013). Here, "ordination" axes, fixed effects and (co)variances provide a complete understanding of ecophysiological data at a glance and the number of latent variables in MHM is chosen automatically through an iterative process (Bhattacharya and Dunson, 2011). Multivariate factor analyses carry a trade-off between using a sufficiently simple model that its parameters can be estimated reliably from available data and using a sufficiently complex model that it can realistically capture the main forms and dimensions of correlation

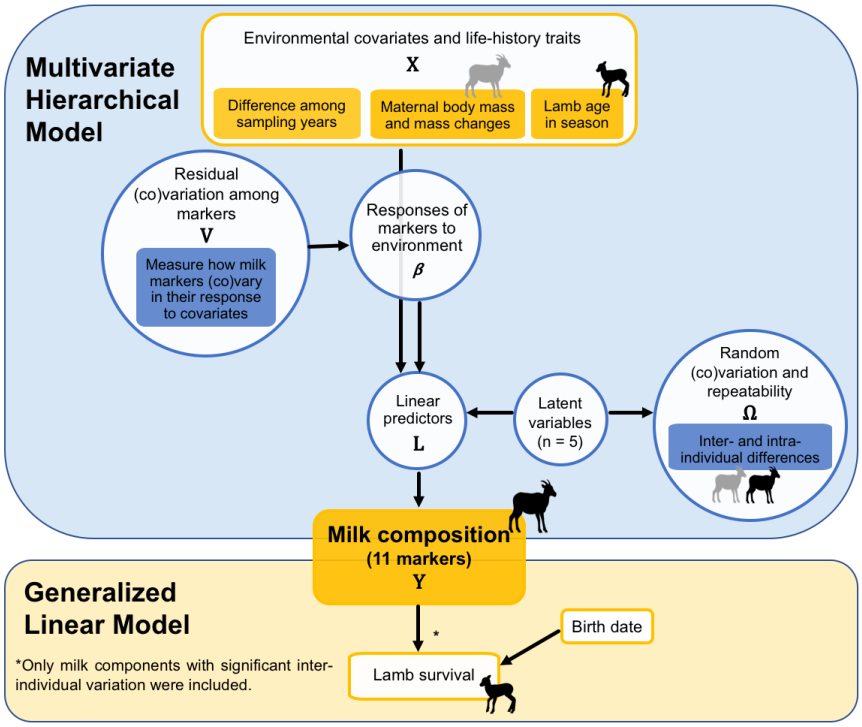


**Figure 3.1 A conceptual summary of the analysis of milk composition in bighorn sheep.**

The analyses were run in two parts : the first part quantified all sources of variation in milk composition using multivariate hierarchical modelling (MHM, upper blue box ; colours in the online version) and included all milk markers ; the second part investigated whether lamb summer survival varied as a function of milk composition using a generalized linear model (GLM, lower yellow box). Only important milk markers were used in the second part of analyses. The orange boxes refer to data, the blue circles to parameters to be estimated, and the arrows to relationships described with the help of statistical distributions. Milk composition—represented by 11 markers in matrix **Y**—is modelled for each ewe sampled over multiple years using a multivariate hierarchical model of **Y** over linear predictors (**L**). The linear predictor has a known variance and statistical distribution. Linear predictors are modelled using fixed and random effects, such that $\mathbf{L} = \beta x$, where $\beta$ represents the response of milk markers to sampling years and life-history traits (matrix **X**). Variation around $\beta$ is captured by the (co)variance matrix **V**. Random terms modelled the variation in milk markers that cannot be attributed to $\beta$. In our study, random effects operate at the interindividual level using the $\mathbf{\Omega}$ (co)variance matrix and at the intraindividual level (not illustrated), after controlling for the covariates in the fixed part of the model. Matrix $\mathbf{\Omega}$ was modelled using five latent variables and intraindividual (co)variation used seven latent variables. The second part of analyses tested whether lamb summer survival varied as a function of milk composition, including only milk components with significant interindividual variation

matrices (Meyer and Kirkpatrick, 2008). While this is also true for joint modelling, the automatic selection of the number of latent variables ensures parsimony and makes joint models much more powerful in highlighting structures in the data than classic multivariate methods.

## 3.4 Materials and methods

### 3.4.1 Study area, population and milk sample collection

The Ram Mountain bighorn sheep population is located in Alberta, Canada (52°N, 115°W, elevation 1,080–2,170 m). Longitudinal monitoring of this population began in 1973 ; lambs, yearlings, adult males and females are captured in a corral trap baited with salt and monitored daily. Lambs are marked during their first summer and their sex and age are recorded with their mother's identity (see Jorgenson et al. (1993) for details on the sampling design). Most births in 1992–2016 occurred in late spring (median date : May 30 $\pm$ 13.4 days), with 80% of lambs born within a 28-day period. Lactation begins at birth, and weaning is assumed to occur around mid-September. Most adult ewes are captured 2–5 times from late May to late September. From 2011 to 2016, milk samples (5–15 ml) were collected in plastic tubes at each capture of a lactating ewe. We adjusted individual mass to 5 June and 15 September (Martin and Pelletier, 2011) ; these dates match the yearly minimum and maximum mass of adults, respectively. To ensure that milk samples were comparable and that females partly sustained the costs of lactation, we only retained mothers whose lamb survived the neonatal period. Overall milk composition was quantified for 221 samples from 34 mothers (3.11 $\pm$ 0.95 samples/mother/year). Presence of colostrum was recorded in $\sim$ 35% of the 34 ewes suggesting we have data from the beginning of the lactation period. Samples were stored at -20°C until laboratory analyses. Milk yield was not quantified and time since last suckling was unknown. All animal-handling procedures were approved by the Animal Care Committee of the Université de Sherbrooke, affiliated with the Canadian Council on Animal care (protocols FP-2016-01 and MFB-2014-01).

Milk fatty acid, protein, glucose and lactose concentrations were quantified using colorimetric methods (Smith et al. (1985) ; Atwood and Hartmann (1992) ; Masuko et al. (2005) ; See Supporting Information Appendix S1 [1]). Milk minerals—calcium (Ca), iron (Fe), magnesium

1. Tous les *Appendix* de ce chapitre sont dans l'Annexe B de la thèse.

(Mg), phosphorus (P), potassium (K), sodium (Na) and zinc (Zn)—were analysed using a two-step technique. First, milk samples were prepared through microwave digestion, which isolated elements of interest in an acid solution (Application Note AM-5 Revision 10-8, CEM Corporation Microwave Sample Preparation Manual). Elemental analysis with inductively coupled plasma-optical emission spectroscopy (ICP ; Perkin-Elmer ICP Optima 4300DV) was then used to isolate the different milk minerals. Fatty acid, protein, glucose and lactose concentrations are expressed as a function of milk volume (mg/ml) while mineral concentrations are given in parts per million (weight or volume ; ppm). We repeated analyses of any sample if the coefficient of variation was 20% or more between replicates, to ensure the microplate reads were reliable. Reads on the ICP were carried out in triplicates and several spectral wavelengths were used to confirm repeatability for each mineral except potassium, for which only one wavelength was available.

### 3.4.2 Maternal traits

In vertebrates, mass is generally positively associated with reproductive success or survival (Stearns, 1992)—but body mass integrates skeletal size and body condition, which may differentially influence reproductive success and respond to different selective pressures (Festa-Bianchet, 1998). Body mass mediates the influence of density and weather on population dynamics of large mammalian herbivores (Bonenfant et al., 2009) but mass changes may better reflect female body condition since it is relative to mass at the beginning of each season (Pelletier et al., 2007). Stored reserves used for reproduction may be better measured by mass changes than absolute body mass, especially in "capital" breeders (Stephens et al., 2009). Bighorn ewes that lost or weaned lambs gained, respectively, 12% and 14% less mass during summer than non-lactating females (Douhard et al., 2018). Maternal winter mass loss is indirectly linked to increased lamb mortality the following winter, through reduced summer lamb growth (a measure of maternal reproductive effort during lactation ; Martin and Festa-Bianchet (2010)). We therefore evaluate the effect of both mass in June and September and mass changes during the previous winter and current summer on milk composition of bighorn ewes.

Our aim was to determine the reproductive consequences of winter mass loss and summer mass gain on milk while accounting for initial mass. We first calculated absolute summer mass gain as the difference between autumn and June mass in year $t$. Absolute winter mass loss was the

difference between June mass in year *t* and autumn mass in year *t* - 1 (Douhard et al., 2018). We then calculated a relative winter mass loss as the residual of a regression between autumn mass in year *t* - 1 and absolute winter mass loss (Douhard et al., 2018). Large, negative values of residuals represent individuals that lost more mass over winter than expected from their body mass the previous autumn. Similarly, we measured relative summer mass gain as the residuals of a regression between June maternal mass and absolute summer gain (Douhard et al., 2018). Relative summer gain and winter loss were thus included as explicative variables in models of milk components (Figure 3.1).

### 3.4.3 Statistical analyses

To investigate the presence of differences between mothers and correlations between physiological markers, we modelled milk concentrations for each macronutrient and mineral using a Bayesian joint modelling approach. Figure 3.2a,b conceptually illustrates the structure of the data. For a milk sample from ewe *i* (out of *n*), *m* milk components were measured. Using these data, we investigated how milk composition was structured at the individual level of biological organization by including a random effect in the model and sampling year as a covariate. As such, we assumed that mother's identity was the dominant factor structuring milk nutrient composition and thus captured among-individual differences. We also modelled residual correlations by including a random term at the sample level (**E** in Figure 3.2c).

Because milk nutrients followed a Gaussian distribution across the samples, the model we used in this study can also be understood as a sophisticated factor analysis. The random terms, accounted for with the one set of latent variables, varied with mother's identity (**H**) and sample (**E**) and were weighted by their associated regression coefficients ($\mathbf{\Lambda}$) (Figure 3.2b). Specifically, the joint model used to characterize milk composition accounts for sampling year while investigating the variance of mother's identity and sample together in the same model using latent variables (Figures 3.1 and 3.2b,c).

Latent variables can be used to model interactions among milk components by assuming that $\mathbf{\Lambda}_{.j} \sim N(0, \mathbf{\Omega})$, where $\mathbf{\Omega}$ is a covariance matrix. It is important to be aware that $\mathbf{\Omega}$ can also be calculated back through $\mathbf{\Lambda}^t\mathbf{\Lambda}$ where *t* is a matrix transpose. The covariance matrix $\mathbf{\Omega}$ can be used to assess how the different milk components are related. However, to make the inter-

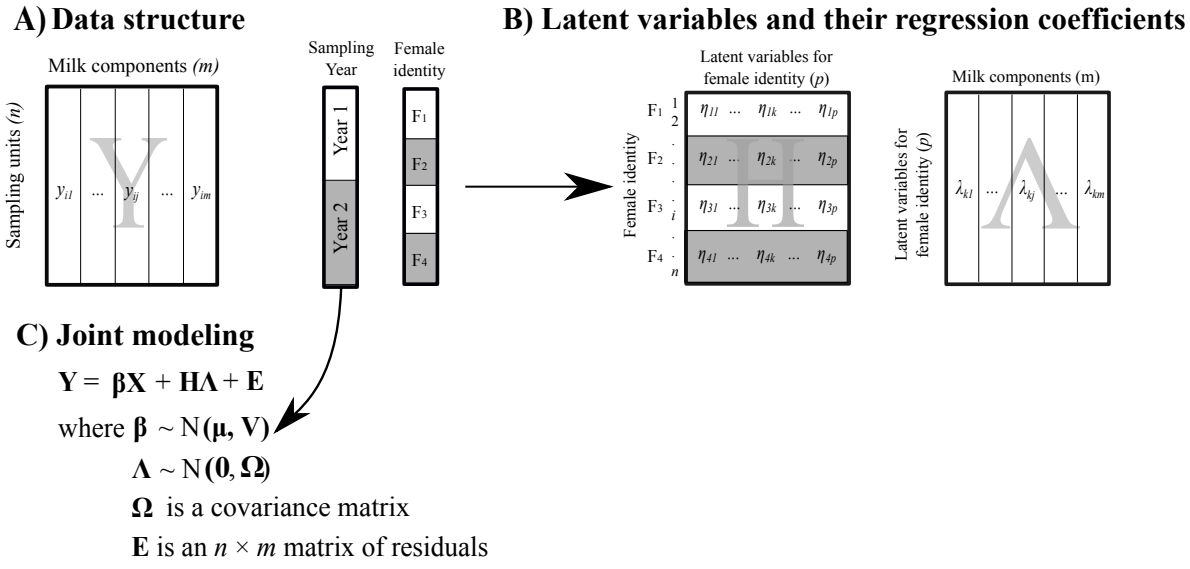


**Figure 3.2 Ecophysiological data used in joint modelling of bighorn ewe milk composition, Ram Mountain, Alberta, Canada, 2011–2016.**

(a) Milk samples (denoted as the **Y** matrix) include milk nutrient concentrations collected from 34 ewes (mothers) over 6 years. Each milk sample represents a sampling unit. Mothers were sampled repeatedly within seasons (F1, F2, . . . ) as conceptually illustrated by the random effect presented on the right of matrix **Y**. (b) In the joint model, the importance of the random effect was quantified using latent variables and their associated regression coefficients to weight the importance of the latent variables. Note that because the same mother was sampled multiple times during the study and through the 6 years sampling period, this redundancy resulted in having the same value associated to either mother's identity for latent variables. This particularity of these latent variables is essential for the model to be constructed. It is caricaturized in the figure by the white and grey blocks in the illustration of matrix **H** , defining mother's identity. (c) The exact definition of the joint model is expressed here in matrix notation, which also includes a random term for the sample-level (residual) variance (**E**). In this modelling approach, the regression coefficients associated to mother's identity (**Λ**) are estimated by controlling for the regression coefficients measuring the importance of sampling year ($\beta$). The structure and number of the latent variables (**H**) were estimated following the procedure described by Bhattacharya and Dunson (2011)

pretation of $\boldsymbol{\Omega}$ easier, we converted it to a correlation matrix with values ranging from -1 to 1. To visualize the relationships among pairs of milk components, a few approaches can be used. In this study, we propose to look at the correlations at the individual (Figure 3.3a) and residual levels (Figure 3.3b) directly. Uncertainties—95% credible intervals—around correlation estimates were also calculated (referred to as "significant" when 95% CI did not span zero ; Supporting Information Figures B.1 and B.2 in Appendix S4).

"Fe" concentration was log-transformed to meet normality assumptions. None of the other response variables diverged enough from normality to be of concern for parameter estimation. We centred and scaled all continuous variables to remove the effect of measurement units. The model parameters were estimated using Markov chain Monte Carlo (MCMC). We carried out 5 runs each with a different set of starting values using 130,000 iterations including 30,000 burn-in iterations ; that is, the first 30,000 iterations were discarded prior to using the remaining 100,000 iterations for estimation and inference (details on the analyses are in Supporting Information Appendices S2 and S3 [2]). We assessed model convergence using the Gelman–Rubin statistics and assumed all parameters converged when the Gelman–Rubin statistic was <1.1. The models were refitted with an additional 100,000 iterations until all parameters of the model converged. All Gelman–Rubin statistics converged after 100,000 iterations (with the additional 30,000 burn-in) ; thus, the parameter estimates presented in this study were calculated from the first of our five runs. All values of the Gelman–Rubin statistics were below 1.08 (see all values in Supporting Information Appendix S3). We carried out all joint modelling analyses with the "HMSC" package (Blanchet et al., 2017) through R version 3.3.3 (R Core Team, 2018). Using the function "Rsquared", we calculated coefficients of determination $(R^2)$ to quantify the explanatory power of both the overall model and each response variable. The upper limit of any of these $(R^2)$ measure is always 1, which corresponds to the ideal case where the model completely replicates the data. Using $(R^2)$ and variation partitioning, we expected to gain a deeper understanding of how yearly and individual variations were important in structuring different milk components. By including mother's identity as a random effect, we partitioned the explained variation in the model between random and fixed effects using the function "vari- Part" of "HMSC". Repeatability (R) was estimated for each trait as the among-individual variance $(V_I)$ divided by total phenotypic variance ($V_P$) (where $V_P$ is the phenotypic variance conditional on the fixed effects, i.e., $V_P = V_I + V_R$) (Nakagawa and Schielzeth, 2010). Prior specification for

2. Ce matériel supplémentaire n'a pas été joint à la thèse. Il s'agit d'un document MarkDown qui détaille toutes les analyses effectuées dans ce chapitre, avec codes, explications et figures, et qui a été inclus avec l'article en ligne. J'ai jugé qu'une telle documentation était longue et peu utile dans le format actuel de la thèse.

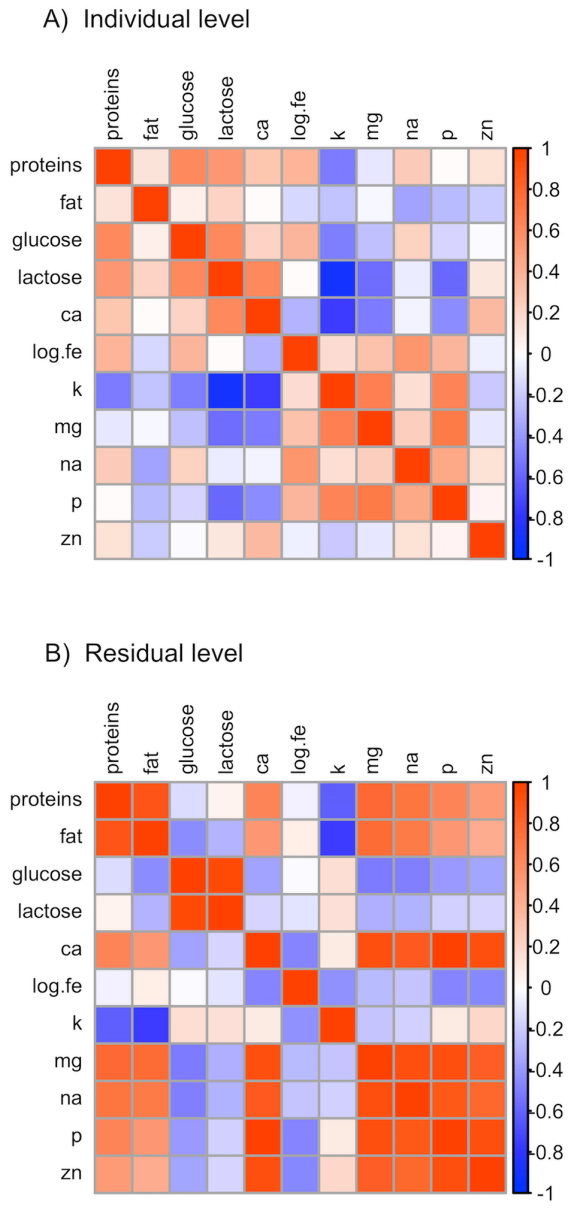


**Figure 3.3 Matrix plots show correlations between pairs of milk components at the individual and residual levels in bighorn sheep, Ram Mountain, Alberta, Canada, 2011–2016.**

Posterior means and quantiles from joint modelling were used. In (a), the matrix represents correlations at the individual level, that is among mothers. In (b), residual (i.e., intraindividual) correlations are illustrated. Blue represents negative correlations and red represents positive correlations between pairs of markers (online version). Correlations are bounded between -1 and 1. Plots were drawn using the "corrplot" function from the "corrplot" R package

the joint model was based on Bhattacharya and Dunson (2011) (see Supporting Information Appendix S2).

Finally, we used results from joint modelling to further explore the effects of life-history parameters on physiology and whether or not milk composition is related to lamb summer survival (Figure 3.1). We investigated the influence of maternal body mass and mass changes on a selected subset of milk parameters for which interindividual variation was detected to test the hypothesis that heavier mothers and mothers affording greater mass changes should have "better" milk than lighter ones. This new multivariate model contained two random effects of mother's and sample identity and included sampling year (a six-level factor), lamb age and one of standardized June or September maternal mass, summer or winter mass changes as fixed effects. We finally modelled weaning probability (0 = lamb died during summer, 1 = survived to weaning) as a function of the subset of milk components to test the hypothesis that, relative to mothers that lost their lambs over summer, mothers that weaned their lamb in mid-September should have a different milk composition. We adjusted three milk components with 30% of interindividual variation—Fe, K, Na—to 50 days after lambing, using linear mixed-effect models, so that milk composition would be comparable among females. Sampling year and lamb age were included as fixed effects, and lamb age was included as a random slope over female identity to account for female's variability in milk composition. We used the resulting adjusted milk markers, with parturition date and sampling year, as explicative variables to test whether milk components affected weaning probability. Summer mortality only occurred in 3 years; thus, a generalized linear model was used since a GLMM did not converge. We carried out all univariate linear mixed-and random-effects models using the "lmer" function available in the "lme4" R package (Bates et al., 2015), and we used the function "confint" to obtain 95% confidence intervals around parameter estimates. We calculated conditional $R^2$ according to Nakagawa and Schielzeth (2013). Parameter estimates are reported as $\beta$ values with their 95% confidence intervals.

## 3.5 Results

The model converged after a total of five and seven latent variables were selected to describe inter- and intraindividual variances, respectively. Coefficient of determination $(R^2)$, averaged across all components, was 0.74. Response variable-specific $R^2$ varied between 0.48 (Fe) and 0.98 (P) (Figure 3.4, Table 3.1 ; see comparison with univariate analyses). Sampling year and mother's identity, respectively, explained, on average across all components, $\sim 29\%$ and $\sim 8\%$ of total variation of the milk composition data after accounting for sampling year as a fixed effect (Figure 3.4). Figure 3.5a illustrates the marginal posterior distribution of parameter estimates of sampling years ; all 95% CI overlapped zero. Intraindividual variation was modelled as the residual variance and explained $\sim 37\%$ of total variation in milk composition. Repeatability estimates (ranging between 0 and 1, 1 being perfectly repeatable) obtained from the joint model, after conditioning on year, ranged from 0.04 (Mg) to 0.64 (K ; mean R = 0.22, Table 3.1, Supporting Information Figures B.3 and B.4 in Appendix S4 [3]). Posterior modes were "distinct" from zero in lactose, Ca, K, Fe, Na and P (Supporting Information Figures B.3 and B.4 in Appendix S4—see the comparison with a GLMM in Supporting Information Figures B.5 and B.6 in Appendix S4).

3. Annexe B dans la thèse.

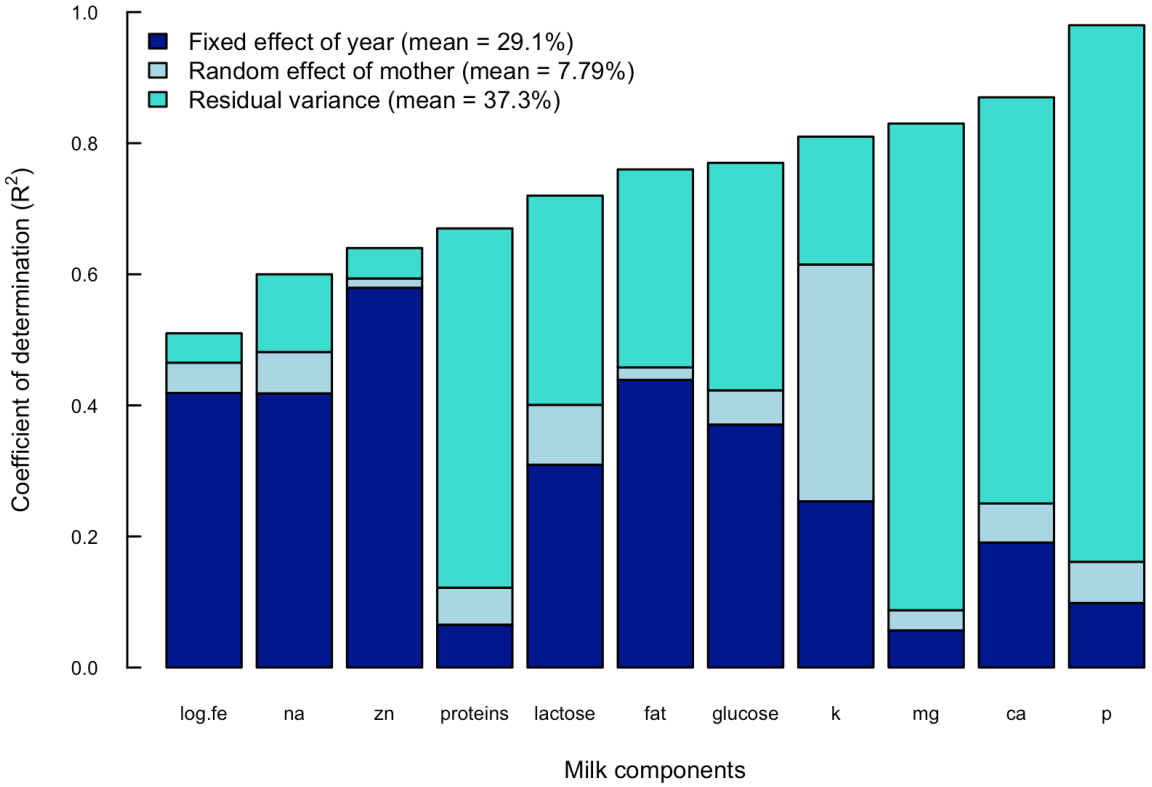


**Figure 3.4 Variance partitioning in milk composition among random and fixed effects in bighorn ewes, Ram Mountain, Alberta, Canada, 2011–2016.**

Each bar corresponds to one milk component and is ordered according to the variable-specific coefficient of determination ($R^2$), in increasing order. Effects of sampling year (fixed effect) are shown in dark blue, while mother effects (random effect) are presented in light blue ; residual variance appears in turquoise (online version). The legend shows mean values over the milk components, multiplied by the proportion of explained variance. The plot was generated using function "variPart" available in the "HMSC" R package (Blanchet et al., 2017)

**Table 3.1 Variance components and coefficient of determination ($R^2$) for bighorn sheep milk composition, Ram Mountain, Alberta, 2011–2016.**

| | Univariate linear mixed effects models | | | | | | Joint modeling | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Milk components | $V_{Individual}$ | 95% CI | $V_{Residual}$ | 95% CI | $R2_{Conditional}$ | R | $V_{Individual}$ | 95% CI | $V_{Residual}$ | 95% CI | R2 MHM | R |
| Proteins | 0.02 | 0.00 - 0.08 | 0.67 | 0.54 - 0.80 | 0.08 | 0.03 | 0.08 | 0.00 - 0.25 | 0.82 | 0.64 - 0.93 | 0.66 | 0.09 |
| Fatty acids | 0.01 | 0.00 - 0.07 | 0.67 | 0.54 - 0.80 | 0.42 | 0.02 | 0.02 | 0.00 - 0.08 | 0.40 | 0.27 - 0.53 | 0.76 | 0.05 |
| Glucose | 0.04 | 0.00 - 0.12 | 0.51 | 0.41 - 0.62 | 0.37 | 0.07 | 0.07 | 0.00 - 0.22 | 0.45 | 0.27 - 0.63 | 0.77 | 0.13 |
| Lactose | 0.05 | 0.00 - 0.14 | 0.55 | 0.44 - 0.66 | 0.32 | 0.08 | 0.13 | 0.02 - 0.30 | 0.45 | 0.22 - 0.65 | 0.71 | 0.22 |
| Ca | 0.02 | 0.00 - 0.06 | 0.34 | 0.28 - 0.41 | 0.24 | 0.05 | 0.07 | 0.01 - 0.18 | 0.71 | 0.59 - 0.83 | 0.87 | 0.09 |
| Fe | 0.05 | 0.00 - 0.13 | 0.51 | 0.41 - 0.61 | 0.44 | 0.09 | 0.09 | 0.01 - 0.27 | 0.08 | 0.00 - 0.38 | 0.48 | 0.56 |
| K | 0.12 | 0.06 - 0.23 | 0.20 | 0.16 - 0.24 | 0.54 | 0.38 | 0.45 | 0.26 - 0.63 | 0.24 | 0.12 - 0.38 | 0.81 | 0.64 |
| Mg | 0.00 | 0.00 - 0.05 | 0.64 | 0.52 - 0.75 | 0.04 | 0.00 | 0.04 | 0.00 - 0.11 | 0.90 | 0.79 - 0.96 | 0.83 | 0.04 |
| Na | 0.01 | 0.00 - 0.01 | 0.05 | 0.04 - 0.06 | 0.47 | 0.10 | 0.10 | 0.02 - 0.24 | 0.19 | 0.10 - 0.31 | 0.60 | 0.34 |
| P | 0.05 | 0.00 - 0.13 | 0.48 | 0.39 - 0.57 | 0.19 | 0.10 | 0.06 | 0.01 - 0.16 | 0.84 | 0.73 - 0.92 | 0.98 | 0.07 |
| Zn | 0.01 | 0.00 - 0.07 | 0.43 | 0.34 - 0.52 | 0.58 | 0.03 | 0.02 | 0.00 - 0.08 | 0.07 | 0.03 - 0.14 | 0.64 | 0.22 |

We used univariate linear random-effects models (left-hand side) and MHM (right-hand side) to calculate variance components. Repeatability (R) was estimated as the among-mother variance $(V_I)$ divided by the total phenotypic variance $(V_P)$ conditional on the sampling year included in the models, that is $V_P = V_{Individual} + V_{Residual}$. Credible intervals and posterior distributions of repeatability estimates are shown in Supporting Information Figures S3 and S4. R2 and R were calculated according to Nakagawa and Schielzeth (2013, 2010), respectively

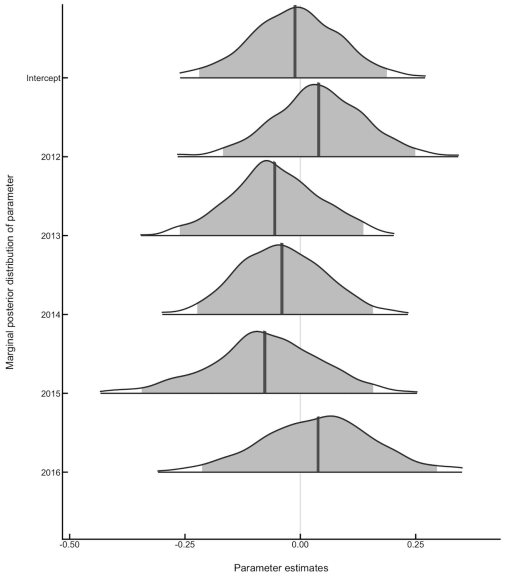


**Figure 3.5 Density plots summarizing the marginal posterior distributions of the regression parameters linking milk composition to sampling year in bighorn sheep, Ram Mountain, Alberta, Canada, 2011–2016.**
Mother's identity and a sample-level factor were included as random effects. The shaded region under the curve represents the 95% uncertainty intervals, and the horizontal line represents 99% of the full marginal distribution (for graphical purpose). The vertical black line represents the mean parameter estimate across all markers. The vertical grey line is drawn on zero for reference. This plot was constructed with the "bayesplot" R package.

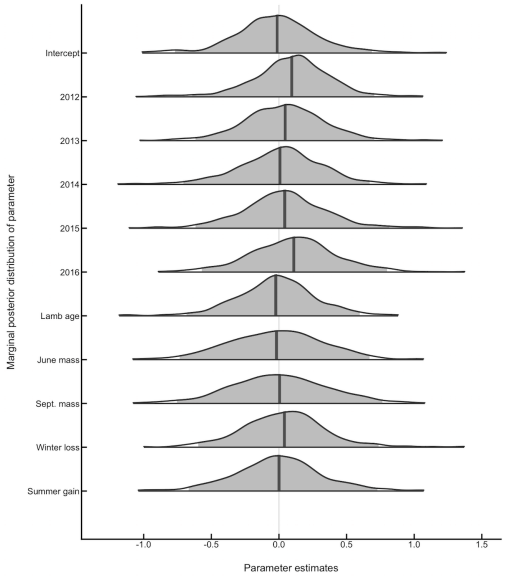


**Figure 3.6 Density plots summarizing the marginal posterior of the regression parameters linking milk composition to sampling year, June and September maternal mass, summer mass gain, winter mass loss, lamb summer survival and lamb age in bighorn sheep, Ram Mountain, Alberta, Canada, 2011–2016.**
Standardized maternal and lamb traits were directly included as fixed effects in the joint model of milk composition. Mother's identity and a sample-level factor were included as random effects. The shaded region under the curve represents the 95% uncertainty intervals, and the horizontal line represents 99% of the full marginal distribution (for graphical purpose). The vertical black line represents the mean parameter estimate across all markers. The vertical grey line is drawn on zero for reference. Detailed parameter estimates and 95% credible intervals for all combinations of response variables and covariates are given in Supporting Information Table S2. This plot was constructed with the "bayesplot" R package.

Multivariate hierarchical modelling of milk composition revealed new associations that were not evident from correlations among raw values (Figure 3.3 and Supporting Information Figure B.7 in Appendix S4). At the individual level, credible intervals around two pairs of correlations did not span zero : Lactose – K (r = 0.88 $[-0.99$ to $-0.49]$) and K – Ca (r = 0.76 $[-0.99$ to $-0.02]$, Supporting Information Figure B.1 in Appendix S4). We identified two major classes of nutrients : macronutrients (proteins, fatty acids, glucose, and lactose), and minerals, which almost always correlated positively with each other, though not significantly (Figure 3.3a). Several residual correlations remained (Figure 3.3b) ; these represented residual (co)variances at the intraindividual level (**E**, Figure 3.2c).

A reduced set of physiological markers was used in our subsequent analyses and included Fe, K and Na concentrations as response variables, which had at least 30% of their explained variance due to mother's identity. Since lactating bighorn ewes gain less summer mass than non-lactating ones (Douhard et al., 2018 ; Festa-Bianchet, 1998), we simultaneously included sampling year, lamb age, standardized June and September mass, and winter and summer mass changes, as fixed covariates in a new joint model of milk composition to investigate whether a female's mass changes correlated with her milk composition (Figure 3.1). Full parameter estimates are reported in Supporting Information Table B.1 in Appendix S4. Effects of June ($\beta_{Junemass} = -0.02[-0.73$ to $0.67]$) and September ($\beta_{Septmass} = 0.00[-0.75$ to $0.76]$) maternal mass on milk composition were negligible (Figure 3.6a). Similar results were obtained when all milk parameters were included as response variables (Supporting Information Table B.2 in Appendix S4).

The lack of effect of standardized maternal June and September mass, and mass changes on milk composition were confirmed by an additional analysis to investigate whether these traits should be quantified as consequences of variation in milk composition. We included maternal variables and milk components as response variables, and sampling year and lamb age as covariates in a new joint model. Heavier mothers in June and September, and mothers showing greater mass gain and greater mass loss than predicted by their initial mass, had increased concentration of fatty acids in their milk (Supporting Information Figures B.8 and B.9 in Appendix S4). Lamb survival to weaning was either not related or marginally related to milk composition ($\beta_Fe = 10.66$ $[-1.92$ to $29.19]$, $\beta_K = -1.28[-4.24$ to $0.81]$, $\beta_Na = -4.74[-12.83$ to $-0.92]$), suggesting little to no impact of milk composition on survival in this sample.

## 3.6 Discussion

Joint modelling allowed us to quantify individual differences in 11 milk macronutrients and minerals jointly with their level-specific correlations, after accounting for annual variation in milk composition. Level-specific correlations are difficult to estimate because small sample sizes and large number of correlated traits often characterize ecological studies. Standard multilevel or mixed modelling approaches facilitate breakdown of means, but not of correlation structures, across hierarchical levels (but see Macciotta et al., 2006). Using this framework, we found that year-to- year variability accounted for approximately one-third of the total variance in milk composition. Importantly, we found small but significant repeatable intermother differences in milk composition. Around 10% of explained variance was due to maternal identity in all components except fatty acid, Mg and P concentrations. Maternal mass and mass changes were positively associated with milk fatty acid concentration, but we found no effect of milk composition on lamb survival to weaning.

Our study reveals little to moderate individual differences in milk composition after accounting for year-to- year variations. Repeatability estimates provided by joint modelling averaged 0.22 across all milk components ; K concentration was the most repeatable component. We suggest that using full information from correlated markers and latent variables to parameterize (co)variance matrices provide increased—and fast—parameter estimates similar to available methods in quantitative genetics (Meyer, 2009 ; Walling et al., 2014). The maternal variance component for fatty acid concentration—and other markers—was systematically low, potentially because unmeasured environmental variables explain most variation in this trait. This is in contrast with studies reporting considerable between-individual variation in milk fat content in wild mammals (e.g., harbour and grey seals ; Lang et al., 2005 ; 2009). Our low sample size—six sampling years—may, however, prevent generalization on a mother's ability to acquire resources and modulate milk composition according to year-to- year variation. One important question is whether or not correlation structure within milk composition would be stable over time. Our repeatability estimates showed a tendency for some milk components to be constant within individuals, but it is also possible that physiology changes as individuals age (Beamonte-Barrientos et al., 2010 ; Nussey et al., 2012). Only continued monitoring and longer datasets would provide better repeatability estimates and would help to assess whether or not milk composition can be qualified using individual covariances between traits.

Individual-level correlations—estimated through five latent variables— among macronutrients and among minerals were mostly positive, while correlations between macronutrients and minerals were mostly negative. Large uncertainties remained around most correlation estimates, and several residual correlations remained unexplained by the model. The only significant associations were lactose – K and Ca – K ; both were negative correlations. What generated these biological associations is unknown, but all these markers were also repeatable among mothers. Temporal variation in nutrient availability partly explains associations among minerals, and between mass gain and minerals found in the forage of migrating caribou (Oster et al., 2018). For instance, P and K are associated with photosynthetic parts and decline as plants senesce, while Ca, Fe and Zn are tied to structural components and may increase in concentration with plant senescence (Oster et al., 2018). Individuals would integrate, through changes in body mass and composition, temporal variation in nutrient content ; associations between milk nutrients could similarly be explained, but perhaps only the most stable macro- or micronutrients would show associations at the individual level.

Despite the link between body mass and condition in bighorn sheep (Festa-Bianchet, 1998 ; Festa-Bianchet et al., 1998), June and September maternal mass, and seasonal mass changes, showed little or no association with milk composition, except when fatty acid concentration and maternal traits where all included as response variables in the joint model. Mothers showing a greater mass gain and winter mass loss than expected by their June and previous September mass, respectively, showed increased fatty acid concentration. Year-to-year variations potentially impacted fatty acid concentration via maternal mass changes, partly explaining positive associations between fatty acid concentrations and maternal traits, and a very low individual variance component in this marker. We acknowledge, however, that the use of residuals to calculate seasonal mass changes—"controlling" for initial mass—may lead to biased parameter estimates and more importantly, omits uncertainty (Freckleton, 2002) around parameter estimates, compared to using multiple regressions. Our effect sizes may be overestimated because correlated variables are used in residual regressions and we thus suggest caution in interpreting biological conclusions. Nevertheless, seasonal mass changes are likely to better reflect associations between a female's body condition and milk composition than maternal mass per se. Our results support studies showing the importance of stored energy to sustain the costs of reproduction, that is "capital" breeding (Festa-Bianchet, 1998 ; Williams et al., 2017). In addition, our results support the contention that females in better "condition" would afford to increase the amount of fat in milk (Mavrogenis and Papachristoforou, 1988 ; Barboza et al., 2009).

The difficulty in detecting strong links between physiology and life-history traits suggests that correlations between milk composition and fitness reported in previous studies (Landete-Castillejos et al., 2001 ; 2005 ; Skibiel and Hood, 2015 ; Quesnel et al., 2017) might be driven by a direct influence of environmental variation and not by a maternal strategy. Our data cannot clearly distinguish whether mothers have no control on the content of their milk or have "flexible" milk composition in face of environmental variation. Milk composition differs among mothers, despite the lack of clear evidence that females with a surplus of energy allocate that energy to milk. Importantly, milk composition showed no association with lamb weaning probability, but we included only females that gave birth and lactated in our analyses. Females unable to produce lambs do not appear in the analyses, which generates an "invisible fraction" in our study, that is individuals subject to viability selection before the trait is measured (Grafen, 1988). Several reasons for not reproducing include low body condition or infertility during conception ; correlations between these traits and future reproductive success may not involve milk composition at all, as analyses of individual fitness are necessarily conditioned on the individuals that are actually observed. It is also possible that females reduce their own survival to maintain lactation and to prioritize their lamb's survival to weaning, similar to age-related resource acquisition and allocation strategies in female Soay sheep (*Ovis aries*, Garnier et al. (2017) ;—but see Festa-Bianchet and Jorgenson (1998) for "selfish" strategies in bighorn ewes). The effects of lactation on lamb survival would thus become difficult to detect unless long-term female survival is also investigated. Finally, perhaps females allocate resources primarily to milk production or are able to produce milk of adequate "quality" to sustain their lambs, regardless of annual variation in the environment.

Joint modelling offers an active avenue to partition variation into spatial, temporal, ecological and/or individual components in physiological traits. Similar to other physiological systems (e.g., the immune system— Pigeon et al., 2013), milk composition may not be captured by one or two components ; the interpretation of multiple markers is challenging due to their large numbers, their level-specific correlations and their variability across temporal and spatial scales. Latent variables are especially useful to extract information from multiple, correlated markers in view of low sample sizes typical of ecological studies. MHM complements multivariate models (e.g., "character state" models ; Houslay and Wilson, 2017 ; Houslay et al., 2018) in unravelling the relative contributions of phenotypic plasticity and adaptive change in physiological and behavioural variation, and could be easily extended beyond the ecophysiology of lactation to other physiological systems. Low repeatability in milk components, and

few correlations at the among-individual level could indicate plastic strategies to cope with varying abiotic or biotic conditions (Buehler et al., 2012) As ecological conditions can influence the degree of individual variation in physiological markers and fitness simultaneously (Graham et al., 2010; Marrot et al., 2015), studies on ecophysiology would gain in assessing first the sources of variability in markers. If the research goal is to link physiological performance (e.g., a physiological "score") to fitness, repeatable markers at the individual level should be used especially if markers are repeatable over the same time frame that fitness variance is being assayed (e.g., lifetime fitness or a period of overwinter mortality). Considering ecological complexity within a single framework should encourage further research to facilitate incorporation of physiological markers with demographic fitness parameters, and to tease apart the relative importance of evolutionary and ecological processes involved in ecophysiological systems.

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## 3.8 Author's contributions

L.-A. R., F.G.B., A.A.C. and F.P. conceived the ideas and designed methodology. L.-A. R. collected the data. F.G.B. and L.-A. R. analysed the data. L.-A. R. led the writing of the manuscript. All authors contributed critically to the drafts and gave final approval for publication.

## 3.9 Data accessibility

Data available from the Dryad Digital Repository https://doi.org/10.5061/dryad.5682v7j(Renaud et al., 2019).

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CHAPITRE 4

# LA COMPOSITION DU LAIT COMME SIGNATURE DES CHANGEMENTS PHÉNOLOGIQUES

*The optimal animal, born with some amount of energy, proceeds through life gaining and expending energy according to some schedule that maximizes its total reproductive output.* Thomas W. Schoener, Theory of Feeding Strategies

## 4.1 Description de l'article et contribution

Avec une augmentation des températures globales, le devancement des dates d'émergence des plantes et de floraison peut allonger les périodes de croissance de la végétation. Les dates de ponte, d'émergence de l'hibernation et de mise-bas sont aussi devancées chez une majorité d'espèces animales. Des changements phénotypiques à différents niveaux trophiques résultent donc de changements phénologiques. Dans ce chapitre je quantifie les causes et les conséquences biologiques de la variabilité interannuelle en survie néonatale, composition du lait, masse au sevrage et survie au premier hiver. Ce chapitre se démarque parce que les études en milieu naturel où il est possible de récolter du lait sur un mammifère (sauvage) sont rares. Ensuite, seul un suivi détaillé comme celui de Ram Mountain permet d'étudier un trait comme la mortalité néonatale. En effet, un suivi régulier des brebis marquées permet la détermination de leur statut de lactation et donc, de leur succès reproducteur. Ce chapitre rassemble donc plusieurs jeux de données originaux incluant des données satellitaires qui ont permis de démontrer l'importance de variables phénologiques à plusieurs stades de vie d'un grand mammifère.

Pour cet article, j'ai planifié les analyses avec Fanie Pelletier. J'ai ensuite effectué la totalité des analyses statistiques et l'écriture de la première version du manuscrit. François Rousseau a extrait les données d'images satellitaires. Fanie Pelletier et Marco Festa-Bianchet ont par la suite commenté plusieurs versions du manuscrit et ont contribué à l'interprétation des données. J'ai participé à la collecte des échantillons de lait et à leur analyse en laboratoire.

**Milk composition in a wild mammal : a physiological signature of phenological changes**

En préparation pour *Proceedings of the Royal Society B : Biological Sciences*

Limoilou-Amelie Renaud, François Rousseu, F. Guillaume Blanchet, Alan A. Cohen, Marco Festa-Bianchet et Fanie Pelletier

## 4.2 Abstract

Understanding how spring phenology influences early life can bring important insights on drivers of future development and survival. We used unique, long-term data from a bighorn sheep population and satellite-derived phenology indices to quantify the relative importance of maternal and environmental influences on neonatal mortality, milk composition and lamb overwinter survival. Over 44 years, 254 of 1372 lambs died at birth (18.5%). Median parturition date differed by 4 days between neonatal survivors and deaths. Short snow-free seasons and long plant growing seasons both increased neonatal survival the following spring. Heavy predation events were associated with reduced neonatal survival, probably through indirect effects on maternal physiology at conception or during gestation. We quantified the match between peak food availability and parturition date, as mismatch with optimal conditions can have detrimental fitness consequences. Only parturition date, not mismatch with spring green-up, affected neonatal survival, suggesting that timing of parturition can have short-term fitness consequences. Based on 221 milk samples from 34 females monitored over 6 years, longer snow-free and plant growing seasons both increased milk fatty acid, iron and lactose concentrations. Structural equation modelling suggested no causality between milk composition, lamb weaning mass and lamb overwinter survival. Our results suggest that spring conditions can have important effects on early developmental stages, survival and milk composition and that large mammals can rely on a strategy intermediate between 'capital' and 'income' breeding when energy demands are high.

**Keyword :**Lactation, maternal effects, neonatal survival, plant phenology, timing of parturition

## 4.3 Introduction

Early-life conditions can have crucial fitness consequences [1]. Parental care is assumed to benefit offspring ontogeny, with effects sometimes lasting through adulthood [1]. Ability to provide parental care, however, may vary according to environmental conditions. Shifts in spring phenology – the timing of key biological events – induced by climate change [2] in temperate environments can increase length of the snow-free period potentially allowing individuals to obtain more resources and provide greater parental care [3]. As a consequence one could expect higher juvenile and adult survival and increased population size [3]. In contrast, failure to synchronize reproductive phenology, starting with timing of parturition, to changes in peak forage availability may have detrimental consequences at the population level, through long-lasting effects on female adult mass and reduced juvenile survival [4]. The timings of key reproductive events in wild animal populations are thus assumed to have evolved to synchronize with seasonal resource availability at lower trophic levels [5] but there is still limited understanding of the relationships between cues and optimal timing, and especially about how these relationships will be affected by environmental changes [6].

Phenological changes are frequently documented in wild organisms in response to climate change [2]. In birds, timing of laying is commonly reported to vary with mean temperatures [7, 8]. Most of these studies, however, have been conducted on passerine birds species which rely on income breeding to meet the energetic costs of reproduction [9, 10]. In those species, nestling feeding is dependent on daily food availability, thus optimal timing of breeding is a critical determinant of future reproductive success and, ultimately, fitness [9]. At the other end of the gradient, species referred to as capital breeders depend mostly on stored body resources [11] to 'finance' reproduction. Among capital breeders, placental mammals such as ruminants increase energy expenditure during lactation up to 4 times their basal metabolic rate [12] and can therefore compensate costs of reproduction by increasing resources acquisition [13]. However if resources are limited, or reproduction is mistimed with the peak in resources abundance, the high energetic costs of parental care can thus lead to trade-offs in life-history traits [14]. A species' position along the capital-income breeding gradient may determine how its life-history traits such as timing of breeding will respond to temporal changes in phenology of its main food resources [15].

To support the high energetic costs of lactation, fitness is assumed to be maximized when

timing of parturition 'matches' vegetation growth [12]. While some studies have investigated the effect of spring phenology on birth timing [9, 16], few evaluated how spring phenology can influence physiological aspects of maternal care in long-lived species. Both maternal reserves and vegetation phenology may affect these traits and influence patterns of neonatal mortality. For example, variation in the amount of milk components early in lactation reflects the amount of maternal reserves in domestic sheep [17].

Neonatal survival can be compromised by suboptimal conditions at parturition, such as low food availability, adverse weather, or high predation rates [18, 19]. For example, interspecific competition among predators (e.g. wolves (*Canis lupus*), bears (*Ursus americanus* and *U. horribilis*) and cougars (*Puma concolor*)) combine with the effects of climate in explaining neonatal survival of elk (*Cervus elaphus*) calves [20]. Direct effects of predation can be detrimental to demography especially when predator specialization is a learned behaviour [21, 22]. Indirect effects of predation can also impact recruitment through dysregulations of female reproductive physiology, resulting from increased female antipredator behaviours and reduced time spent foraging ([23], but see [24] for contrasting results).

Here, we investigate the relationships between milk nutrients and their associations with timing of parturition, plant phenology and offspring traits in a natural population of ungulates to test the hypothesis that milk composition, neonatal survival and lamb overwinter survival are affected by spring phenology. Milk plays a crucial role in offspring development [25]. In the first hours of life, immunoglobulins in colostrum boost the newborn immune system of bovine mammals [26]. Milk also contains other important nutrients such as minerals that, together with immunoglobulins, enzymes and hormones, enhance offspring immunity, growth and development [27, 28]. Studies in primates [29], marine [30] and domestic mammals [28] have established that milk production and composition are important for juvenile growth, weaning mass and survival which in turn increase population growth rate by affecting recruitment [3]. Fewer studies, however, have asked how change in spring phenology affects milk composition and whether it correlates with offspring growth and survival in terrestrial wild eutherians (but see [31, 32] for examples on marsupials). Thus, much of our current knowledge is based on short-term studies [33, 32] focusing mainly on measured fatty acid and milk protein content. In bighorn sheep (*Ovis canadensis*), milk composition can be highly variable from year to year [34] but the concomitance of plant phenology and maternal body condition for inter-individual and inter-annual differences in milk composition, including minerals, is unknown.

In this study, we evaluate the relative importance of maternal reserves and vegetation quality for lamb neonatal survival and milk composition changes through the course of lactation. We specifically aim to 1) quantify the link between spring phenology and parturition date and its importance for neonatal survival ; 2) investigate how spring phenology drives variation in milk composition ; and 3) assess how milk composition influences weaning mass and overwinter survival, two important fitness-related traits in our model species [35, 36]. We recently found that milk composition is strongly affected by inter-annual environmental variations and, to a lesser extent, by individual differences among mothers [34]. Building on those findings, we hypothesize that inter-annual variations in neonatal survival at the population level, and milk composition at the individual level, can be explained by spring phenology. Neonatal survival is rarely available from studies in the wild, but can be a critical component of population growth [37]. To evaluate the effect of variation of milk composition on fitness, we further hypothesized that major milk macronutrients – fatty acids, proteins and glucose – indirectly affects lamb overwinter survival through effects on weaning mass. This study uses detailed individual data to answer fundamental questions on how spring phenology influences early-life survival in a wild vertebrate.

## 4.4 Methods

### 4.4.1 Field and laboratory techniques

The Ram Mountain bighorn sheep population in Alberta, Canada (52°N, 115°W, elevation 1080 – 2170 m) has been monitored since 1971. Sheep are captured in a corral trap baited with salt and observed daily. Most lambs are marked during their first summer and their sex and age are recorded along with maternal identity (see [38] for details on sampling design). Weaning occurs in mid-September ; later suckles are rare and very short [39]. We document a neonatal mortality when a female shows evidence of lactation at first capture but is never seen with a lamb in the following days. For lambs that survive the neonatal stage, parturition date was estimated from 1992 to 2017 (except for 1993) based on morphological observations, including lamb size, presence of the umbilical cord and dark grey coat [40]. Ewes isolate themselves for 2-3 days post-partum before they join other ewes [39].The number of days since an ewe was last seen before giving birth helped us estimate lamb birthdate.

For lambs that died during the neonatal stage, we estimated birth dates based on a combination of lactation status of the mothers and when each ewe isolated itself to give birth. Females start to produce colostrum before parturition but produce 'watery' milk following lamb death and finally stop producing milk. These changes in lactation status between captures helped us estimate parturition date of lambs that died at or near birth. We assigned a parturition date only when uncertainty around parturition date was less than 10 days (n = 57 parturition dates on a total of 254 neonatal mortalities). We refer to these dates as 'parturition dates for neonatal deaths' since they were estimated with less certainty than those for lambs that were seen ('survivors'). Detailed female capture dates and information on lactation status were only available after 1994.

Most adult ewes are captured and weighted 2 to 5 times each summer, from late May to late September. We adjusted all individual female mass to June 5 and September 15, and individual lamb mass to June 15 and September 15 [41]. From 2011 to 2016, we collected milk samples (5-15 mL) from ewes at each capture and samples were stored at -20 °C until laboratory analyses. We quantified milk composition for 221 samples from 34 mothers of 76 lambs (1.74 $\pm$ 2.53 samples/mother/year). We could not quantify milk yield nor time since last suckling. We estimated milk fatty acid, protein, glucose and lactose concentrations as described in [34]. Briefly, we quantified macronutrients using colorimetric methods while minerals – calcium (Ca), iron (Fe), magnesium (Mg), phosphorus (P), potassium (K), sodium (Na) and zinc (Zn) – were analyzed by inductively coupled plasma-optical emission spectroscopy (ICP; Perkin-Elmer ICP Optima 4300DV). Fatty acid, protein, glucose and lactose concentrations are expressed as a function of milk volume ($mg{\cdot}mL^{-1}$). Mineral concentrations are reported in parts per million (weight or volume; ppm). To investigate if milk composition affected lamb weaning mass and overwinter survival, we calculated the energy derived from fatty acids, protein and glucose concentrations per unit volume. We assumed that energy values were 24.6 kJ $g^{-1}$ for protein, 38.1 kJ $g^{-1}$ for lipid and 16.1 kJ $g^{-1}$ for glucose [42].

### 4.4.2 Extraction of phenology indices

We used data acquired by the Moderate Resolution Imaging Spectroradiometer (MODIS) [43] to quantify plant phenology throughout the sampling region in autumn and spring seasons (Table C.1).We extracted Julian dates (1 Jan = 1 JD) of the maximal increase and decrease in

seven time series values (Table C.1). We refer to these as 'green-up' or 'green-down' dates for spring and autumn phenologies, respectively. 'Green-down' dates, i.e., vegetation senescence dates, were used to calculate a growing season length : the number of days between green-up and green-down, hereafter referred to as 'Season length'. For snow, dates represent the maximal rate of change in the probability of snow on the ground. We refer to spring dates as 'Snowmelt date' and to autumn dates as 'Snowfall date'. The season length based on snow cover was the difference, in Julian days, between 'Snowfall date' and 'Snowmelt date', hereafter referred to as 'Snow-free' season.

Most phenology indices were correlated to each other. To reduce the number of variables in our analyses, we conducted a two-step analysis. We first conducted a principal component analysis including all extracted phenology variables to investigate which indices contributed the most to principal components. To do so, we used factor loadings to select indices that contributed the most to the first and second principal components (details in Supplementary Information). According to correlations among raw variables (Table C.2) in combination with unscaled variables' loadings on each PC (Table C.3), we selected two indices that best described spring phenology : dates of gross primary productivity (GPP i.e., green-up) and snowmelt dates (Table C.3) in analyses of neonatal survival and milk composition.

### 4.4.3 Neonatal survival and parturition dates

We first investigated whether a temporal trend in neonatal survival in our study population coincides with temporal trends in phenology indices. To do so, we calculated the annual proportion of lambs that survived the neonatal stage over the total number born (see Table 4.1 for a summary of analyses). We used a logistic regression to investigate temporal trends by regressing neonatal survival over years. Similarly, we used linear regressions to investigate temporal trends in dates of GPP and snowmelt.

**Table 4.1 Summary of analyses of bighorn lamb neonatal survival at the population and individual levels, Ram Mountain, Alberta, 1972-2016.**

| **Population-level analyses** | | |
|---|---|---|
| *Temporal trends in neonatal survival and phenology indices* | | |
| Response variable | Explicative variables | Test |
| Neonatal survival (dead / dead+live, n = 254 ) | Year | Logistic regression |
| Date of gross-primary productivity (GPP; i.e. green-up, n = 19) | Year | Linear regression |
| Date of snowmelt (n = 19) | Year | Linear regression |
| Season length (GPP green-down – GPP green-up date, n = 19) | Year | Linear regression |
| Season length (snowfall – snowmelt dates, n = 19) | Year | Linear regression |
| *Determinants of neonatal survival : green-up and snowmelt dates* | | |
| Neonatal survival (dead/dead+live) | Adult female density ($N_t$) | Logistic regression, binomial family |
| | Previous adult female density ($N_{t-1}$) | |
| | Predation (high or low) | |
| | Green-up date | |
| | Snowmelt date | |
| | Green-up date x $N_t$ | |
| | Snowmelt date x $N_t$ | |
| | Green-up date x $N_{t-1}$ | |
| | Snowmelt date x $N_{t-1}$ | |
| *Determinants of neonatal survival : season lengths from GPP and Snow indices* | | |
| Neonatal survival (dead/dead+live) | $N_t$ | Logistic regression, binomial family |
| | $N_{t-1}$ | |
| | Predation (high or low) | |
| | GPP season length | |
| | Snow-free season length | |
| | GPP season length x $N_t$ | |
| | Snow-free season length x $N_t$ | |
| | GPP season length x $N_{t-1}$ | |
| | Snow-free season length x $N_{t-1}$ | |
| **Individual-level analysis** | | |
| *Determinants of neonatal survival (including all female observations)* | | |
| Neonatal survival (0 = died, 1 = survived, n = 397) | Maternal mass the previous autumn | Logistic regression, binomial family |
| | $\Delta$ (parturition date – green-up date) | |
| | $N_t$ | |
| | $N_{t-1}$ | |
| | Predation (high or low) | |

Possible determinants of neonatal survival included spring phenology, adult female density (number of females aged $\leq 2$ years) and predation, which has been suggested to be either weak or strong in years with heavy cougar predation [22]. In this study, predation was measured the previous year to account for lagged responses in neonatal survival. We used logistic regressions with a binomial error distribution to test the effect of adult female density in the current ($N_t$) and previous year ($N_{t-1}$), predation, green-up and snowmelt dates and current and previous growing season length ($Year_{t-1}$) on neonatal survival, and all two-way interactions with current and previous density (Table 4.1). Density was included as a continuous variable. We did not include sampling year since neonatal survival was measured at the population level.

Phenology dates and season lengths were not included in the same models because these variables were correlated (r = -0.93 for green-up date and season length and r = -0.82 for snowmelt dates and season length). We thus tested significance of spring phenology dates and season lengths in separate models using a backward stepwise model selection. Final models for each set of variables were determined by sequentially removing the term with the highest *P*-value until all terms were significant. Significance of each model term was then assessed with an analysis of deviance between nested models, tested against a chi-square distribution with degrees of freedom that corresponded to the difference in the number of terms estimated. We report estimates and lower and upper 95% confidence intervals (i.e. slope [LCI – UCI]).

To test the hypothesis that lambs born before or close to the date of green-up would have a better survival relative to lambs born after the date of green-up (a 'mismatch'), we tested whether the deviation, in days, between parturition dates and annual dates of green-up influenced neonatal survival. Negative deviation values would indicate early births relative to green-up. This analysis included all individual observations so that adjusted female mass in previous September and 'absolute' parturition date could be modelled as predictors of neonatal survival (Table 4.1). Neonatal survival was tested as an individual measure – whether a female gave birth to a lamb that died or survived – or 'relative' to the population average by subtracting yearly population means to individual observations. We modelled neonatal survival using a logistic regression with a binomial error distribution and 'relative' neonatal survival as a linear regression with a gaussian error distribution. We included all available parturition dates (neonatal deaths and survivals) in this dataset. A mixed-effects model would not converge because of small number of measurements per female, but slope estimates were identical to those of the logistic regression.

### 4.4.4 Multivariate hierarchical modeling of milk composition

Our next analysis aimed at decomposing the multiple sources of variation in milk composition using a multivariate framework suitable for the analysis of ecophysiological data [34]. This analysis used individual-level observations to include all repeated female observations within and over years. We used Bayesian Multivariate Hierarchical Modeling (MHM, [44]) to investigate the joint responses of milk components to maternal traits, lamb traits and spring phenology. We tested the effects of spring phenology dates and season lengths in separate models. To ensure that the milk sampling period matched that of phenology dates, we only included milk samples collected before Julian day 170, the latest green-up recorded (mean sample/female $\pm$ SD = 1.46 $\pm$ 0.59). Based on [34] and to enhance detection of phenology effects on milk composition, we selected a subset of nutrients for which interannual variance was $\geq$ 30% of the total explained variance, which is equivalent to the average variation explained across all nutrients in a former analysis of milk composition [34].

We modeled this subset of milk components by assuming a Gaussian error, as for [34]. We accounted for sampling year in the model as a random effect since we were interested in interannual variance in milk composition. We also included maternal identity and milk sample number as random effects to respectively model inter- individual and residual (within-individual) variances. Sample number accounted for variation in milk composition that could not be modeled using female identity or sampling year ; it thus represents the variation that a female expresses over its repeated milk measurements within seasons or over years. Maternal traits included summer mass gain and winter loss (controlled for June mass and previous September mass, respectively, because changes depend on initial mass [35]). We included age (days since estimated birth), lamb sex and birth date as lamb traits ; sex was dropped from the model because it showed no effect on milk composition.

Iron concentration was log-transformed to meet normality requirements. All other response variables were normally distributed. Prior to analyses, we centered and scaled all continuous variables to remove the effect of measurement units. The model parameters were estimated using Markov chain Monte Carlo (MCMC). The model estimations were performed using 260,000 iterations including 60,000 burn-in iterations and a thinning of 200, for a total of 1000 remaining iterations for estimation and inference. We assessed model convergence using the Gelman-Rubin statistics (converged at < 1.1). We quantified the explanatory power of mo-

dels with a coefficients of determination ($R^2$), using the function 'Rsquared' from 'HMSC' R package [45], for each response variable and for the multivariate response, which is calculated as the average ($R^2$) over all response components. Prior specification for the model followed the ones proposed by [46] for the latent variables and [47] for the fixed explanatory variables. We presented parameters estimates using 95% credibility intervals (i.e. slope [LCI – UCI]).

### 4.4.5 Path analysis of lamb weaning mass and overwinter survival

Lamb age (in days) and sampling year influence milk composition [34]. We adjusted each milk component by including both lamb age (continuous) and year (factor) as fixed variables in a linear mixed-effects model for each milk component. We included a random slope of lamb age over mother identity in all models. We were interested in how mothers allocated energy to very young lambs that survived the neonatal stage. Using the beta-coefficients of these mixed regressions, each milk component was thus adjusted to a lamb age of 10 days to approximate milk composition during early lactation. We then calculated the energy derived from adjusted milk macronutrients in kJ $g^{-1}$ per unit volume (mL), assuming that energy values were 24.6 kJ$g^{-1}$ for protein, 38.1 kJ $g^{-1}$ for lipid and 16.1 kJ $g^{-1}$ for glucose [42].

To disentangle the direct and indirect effects of energy derived from adjusted milk components on lamb weaning mass and overwinter survival over 6 years, we analyzed the datasets on adjusted milk energy content, phenology, lamb weaning mass, and overwinter survival (0 = died or 1 = survived) using Piecewise Structural Equation Modeling (SEM, n = 65) [48]. Piecewise SEMs are conceptually similar to classical path analysis but solve each component model separately. Thus, piecewise SEM allows for models with their own sampling distributions and small sample sizes [48]. We first fit the three component models (response variables : milk energy content, lamb weaning mass and overwinter survival) as generalized linear-mixed effects models (GLMMs) with their appropriate error distributions (Gaussian and binomial for overwinter survival). Year is directly correlated to annual phenology values ; to avoid variance estimation problems, we included it as a random effect instead of modelling its average effect. Each component model had maternal identity and sampling year (thirty and six levels, respectively) as random effects to account for non-independence of observations within years and mothers.

All three models tested for an effect of parturition date on milk composition and lamb weaning mass (Figure 4.6). The relationship between weaning mass and parturition date varies by lamb sex and male lambs born late have lower survival than female lambs [49]. The direct paths between parturition date and milk energy and between lamb sex and milk energy tested whether, independently of green-up date, parturition date and lamb sex influenced milk energy content. The direct path between parturition date and lamb weaning mass tested whether, given equal energy content (per mL), late-born lambs would be lighter in September and have lower survival than early-born lambs. Given equal energy content, we expected male lambs to be heavier than female lambs. We hypothesized that spring phenology would be important at all stages of lamb growth but since milk component were adjusted at 10 days of lamb age, we only included green-up date in our models. We expected that a late green-up would reduce milk energy, independently of parturition date, by forcing females to use body stores for early lactation. A late green-up would leave little time for lambs to benefit from forage availability before and after weaning, thus resulting in a lower weaning mass and winter survival. We thus initially tested a direct effect of green-up date on all response variables, but final SEMs are illustrated in Figure 4.6.

We compared three SEMs to determine the key direct and/or indirect effects of milk energy on lamb overwinter survival. Our first path model, or 'null' model (Figure 4.6a), tested the prediction that lamb overwinter survival was independent of any variable included in the causal model. Our second path model (Figure 4.6b), tested the prediction that milk composition during lactation indirectly affects lamb survival by increasing lamb weaning mass. Model 3 (Figure 4.6c) tested whether milk composition also directly affected survival through mechanisms other than mass gain, for example by increasing immunity, bone and motor development and other physiological systems. We used d-separation [50] to test whether each SEM fit was improved by the inclusion of identified missing paths.The d-separation test generates a Fisher's C test statistic, which can be used to assess overall fit of the SEM and to calculate Akaike's information criterion corrected for small sample sizes (AICc) for model selection [50] . As we were mostly interested in the direct or indirect effect of milk on survival, we compared the three candidate models and selected the SEM with the lowest AICc value. For the best-fit SEM, we extracted all coefficients to determine the strength of paths. We conducted all statistical analyses in R version 3.5.1 [51]. We used the R package 'piecewiseSEM' [48] for d-separation tests and report estimates, standard errors and associated *P*-values as given by the path models coefficients.

## 4.5 Results

From 1972 to 2016, we recorded 254 neonatal mortalities over 1372 births, an overall rate of 18.5%. This rate was 19.6% between 2001 and 2016, the subset of years including phenological variables. Over 45 years, we found a slight but significant decrease in neonatal survival, but large year-to-year variations ($\beta_{year}$ = -0.023 [-0.026 to -0.020], Figure 4.1a). Median parturition date was June 2 $\pm$ 10.9 SD for 57 lambs that died during the neonatal stage and it was May 30 $\pm$ 13.9 SD for 397 lambs that survived (Figure 4.1b).

From 2001 to 2016, mean date of green-up was May 27 $\pm$ 15.88 SD (median : 151 Julian day, figure 4.1c) and mean snowmelt was April 30 $\pm$ 14.77 SD (median : 124 Julian day). Growing season length averaged 102.41 days $\pm$ 20.78 SD based on GPP and 166.06 days $\pm$ 18.81 SD based on snow cover. There was no significant trend in green-up (slope = -0.990 [-2.633 – 0.653]) or snowmelt dates over years (slope = -0.780 [-2.187 – 0.627]); a quadratic effect of year gave similar results. All estimates and corresponding SE and *P*-values are also given in Table C.4.

### 4.5.1 Influence of spring phenology on neonatal survival : green-up date and length of previous growing season

Phenology dates and length of previous growing season both influenced neonatal survival. Final model of phenology dates included snowmelt date in interaction with current density, and predation the previous year. At low population density, neonatal survival increased with later snowmelt, but the effect was reversed at high densities (-0.415 [-0.845 – -0.010], Figure 4.2a). High predation in the previous year reduced neonatal survival in year$_t$ (-2.268 [-3.444 – -1.172], Figure 4.2b). Final model of season lengths included previous snow-free season lengths, previous season length from GPP and previous predation. Previous snow-free season length reduced neonatal survival (-0.427 [-0.831 – -0.036]), and previous season length (GPP) increased it (0.459 [0.048 – 0.924], Figure 4.3). Neonatal survival was also lower in years following high predation levels (–2.138 [-3.029 – -1.286]). The ‘phenology dates’ and ‘season lengths’ models respectively explained 66.7% and 84.2% of the variance in neonatal survival.

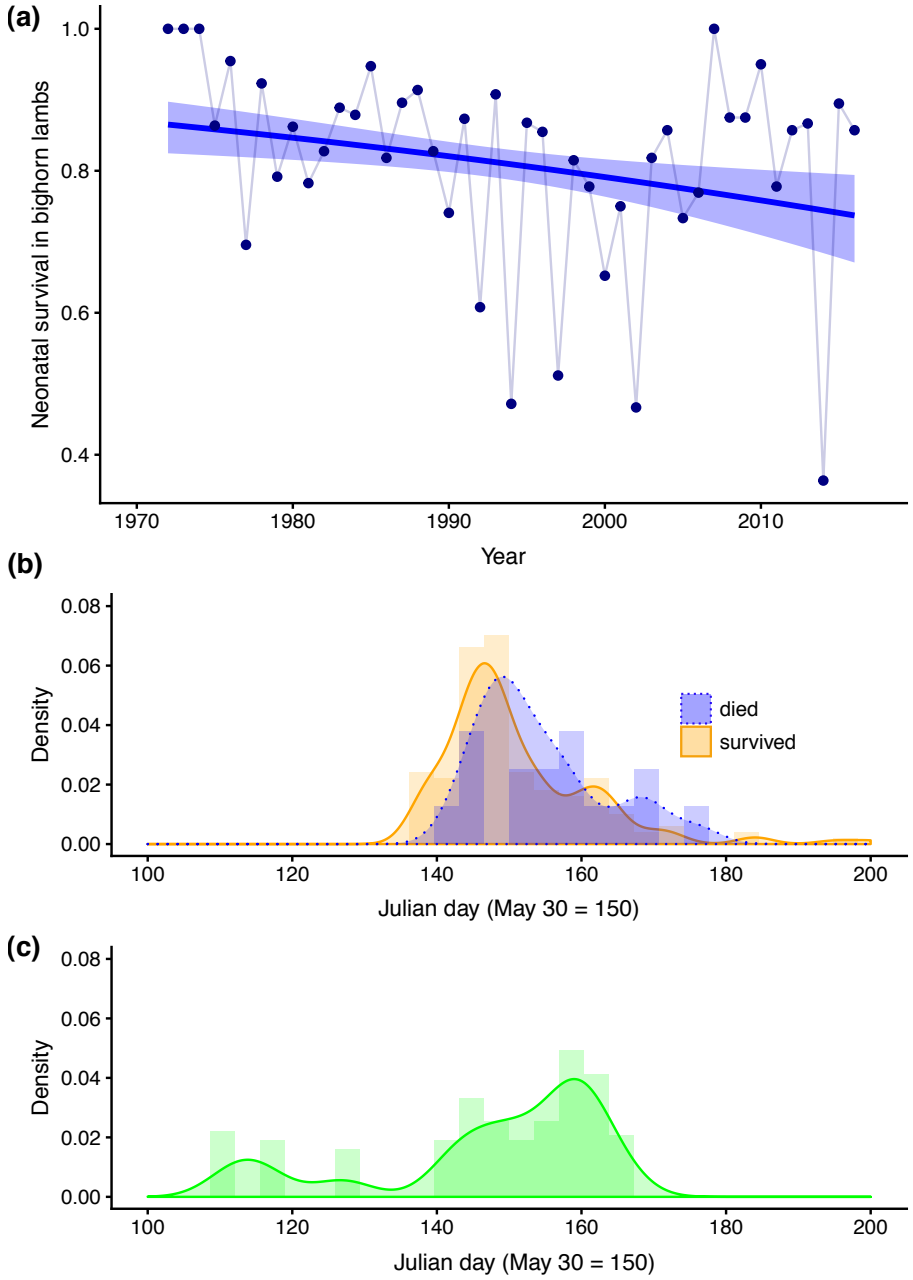


**Figure 4.1** ***(a)*** **Temporal trend in neonatal survival in bighorn sheep lambs, Ram Mountain, Alberta, Canada, 1971 – 2016 (n = 254 neonatal deaths over 1372 lambs).** ***(b)*** **The density distribution of lambs that survived (orange, solid line) and that died (purple, dotted line) during the neonatal stage as a function of parturition date (1 Jan = 1).** ***(c)*** **The density distribution of green-up dates as extracted from gross-primary productivity time series.** Julian day 150 is equivalent to May 30 in non-leap years. In *(a)*, the blue thick line represents the predicted effect of year on neonatal survival reported in the text.

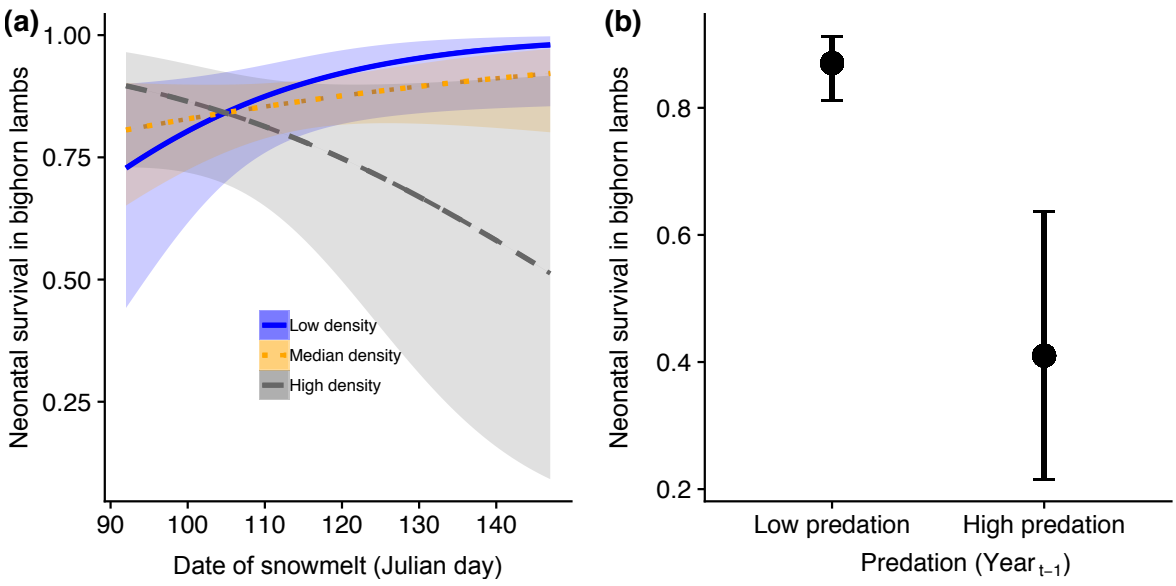


**Figure 4.2 The effects of *(a)* the interaction of date of snowmelt and adult female density and *(b)* predation the previous year on bighorn lamb neonatal survival, Ram Mountain, Alberta, Canada, 2000 – 2016 (n = 290)**

In *(a)*, lines represent the minimum = 16, median = 22, and maximal density = 32 for graphical purpose but density was included as a continuous variable in the models. 95% confidence intervals around estimates are shown in *(b)*

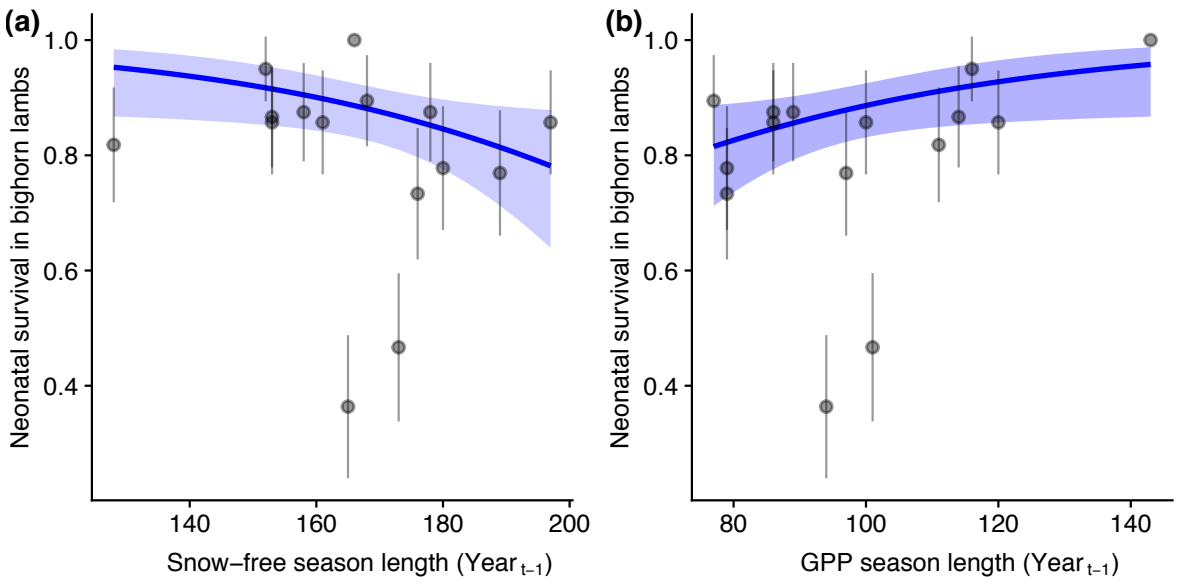


**Figure 4.3 The effects of *(a)* previous 'snow-free' season length and *(b)* previous GPP season length on neonatal survival in bighorn sheep lambs, Ram Mountain, Alberta, Canada, 2000 – 2016 (n = 290).**

Points represent proportions ± SE. SNOW : maximum snow extent; GPP : Gross primary productivity

Using individual data, we detected no significant influence of the interval, in days, between parturition date and green-up date on neonatal survival, either as a linear or quadratic variable. The final model included only a linear effect of parturition date and maternal mass the previous fall. Late parturition date (in 'absolute' days) decreased neonatal survival (-0.037 [-0.070 – -0.004], Figure 4.4); its effect was however negligible on 'relative' neonatal survival (-0.003 [-0.008 – 0.002], Table C.4). Neonatal survival decreased with maternal mass the previous autumn, but the effect was not significant (-0.138 [-0.636 – 0.329], Table C.4).

### 4.5.2 Causes of variation in milk composition

On average, the model testing spring phenology dates explained 77.4% of variation in milk composition (range of $R^2$ across milk components = 0.67 – 0.87) but effects of green-up and snowmelt dates were not different from zero across model iterations and milk markers (Figure C.1a), including fatty acids (Figure 4.5a). In the model testing growing season lengths, $R^2$ was 0.64 (range 0.58 – 0.68). Phenology affected fatty acids ($slope_{GPP}$ = 0.22 [0.02 – 0.44], $slope_{SNOW}$ = 0.44 [0.15 – 0.68], Figure 4.5b), Fe (0.59 [0.28 – 0.87]) and lactose (0.31 [0.06 – 0.55). Later parturition date was associated with lower Na concentrations (-0.16 [-0.28 – -0.03]) and higher fatty acid concentrations (0.16 [0.06 – 0.27], Figure 4.5b). Maternal mass changes had no effect on milk composition.

### 4.5.3 SEMs of lamb weaning mass and overwinter survival

Hypotheses 1, 2 and 3 were not rejected by d-separation suggesting that the data supported the implied independences for these three causal models [50]. We therefore compared the simplified models with AIC. Direct paths between green-up date and lamb weaning mass and survival were dropped from all models. Final models 2 (Figure 4.6b) and 3 (Figure 4.6c) had respective AICs of 15.864 and 13.231 higher than model 1 ('null' model, Figure 4.6a, AIC = 33.026). Lamb weaning mass was mostly influenced by the direct effects of parturition date and lamb sex, and marginally by milk composition (slope = 0.290, $P$-value = 0.096). Male lambs were heavier than females at weaning, independently of parturition date. Both late green-up and early parturition were associated with low milk energy content.

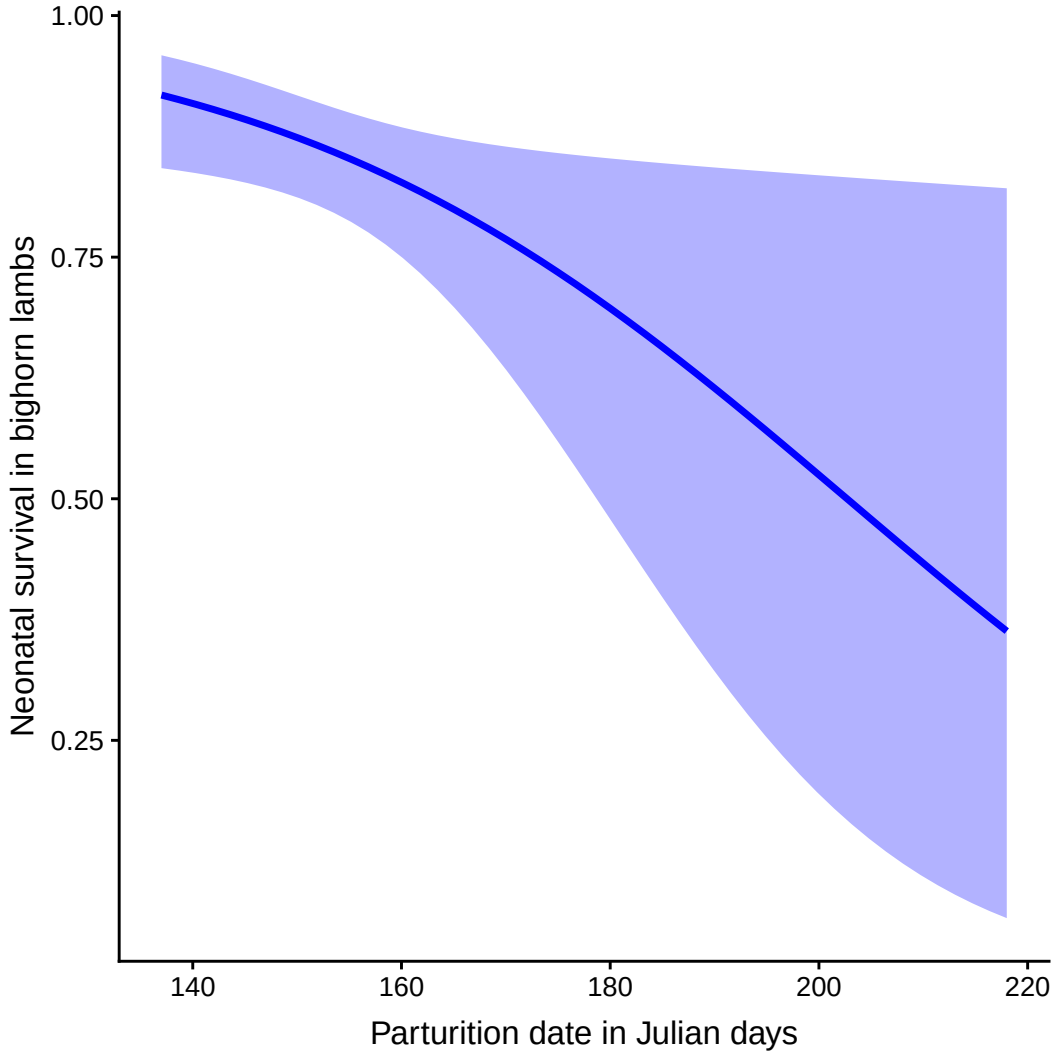


**Figure 4.4 The effects of parturition date, in Julian days, on neonatal survival in bighorn sheep lambs, Ram Mountain, Alberta, Canada, 2000 – 2016 (n = 397).**

The line represents predictions from the fitted model.

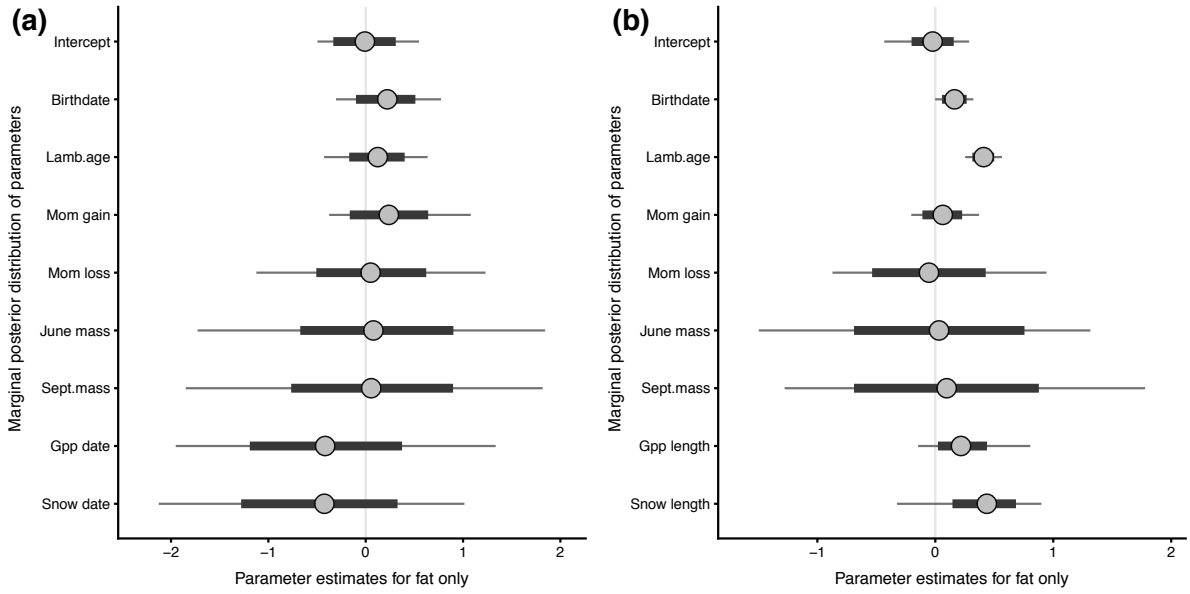


**Figure 4.5 The marginal posterior distributions of regression parameters linking milk fatty acid concentrations to spring phenology and maternal covariates in bighorn sheep, Ram Mountain, Alberta, Canada, 2011– 2016 (n = 220).**

*(a)* Dates of snowmelt and green-up date (GPP); *(b)* 'Snow-free' season length and GPP growing season length. Sampling year, mother's identity and a sample-level factor were included as random effects. The thick horizontal line represents the 95% uncertainty intervals, the thin horizontal line, the full marginal distribution and the grey dot, the mean. The vertical grey line is drawn on zero for reference

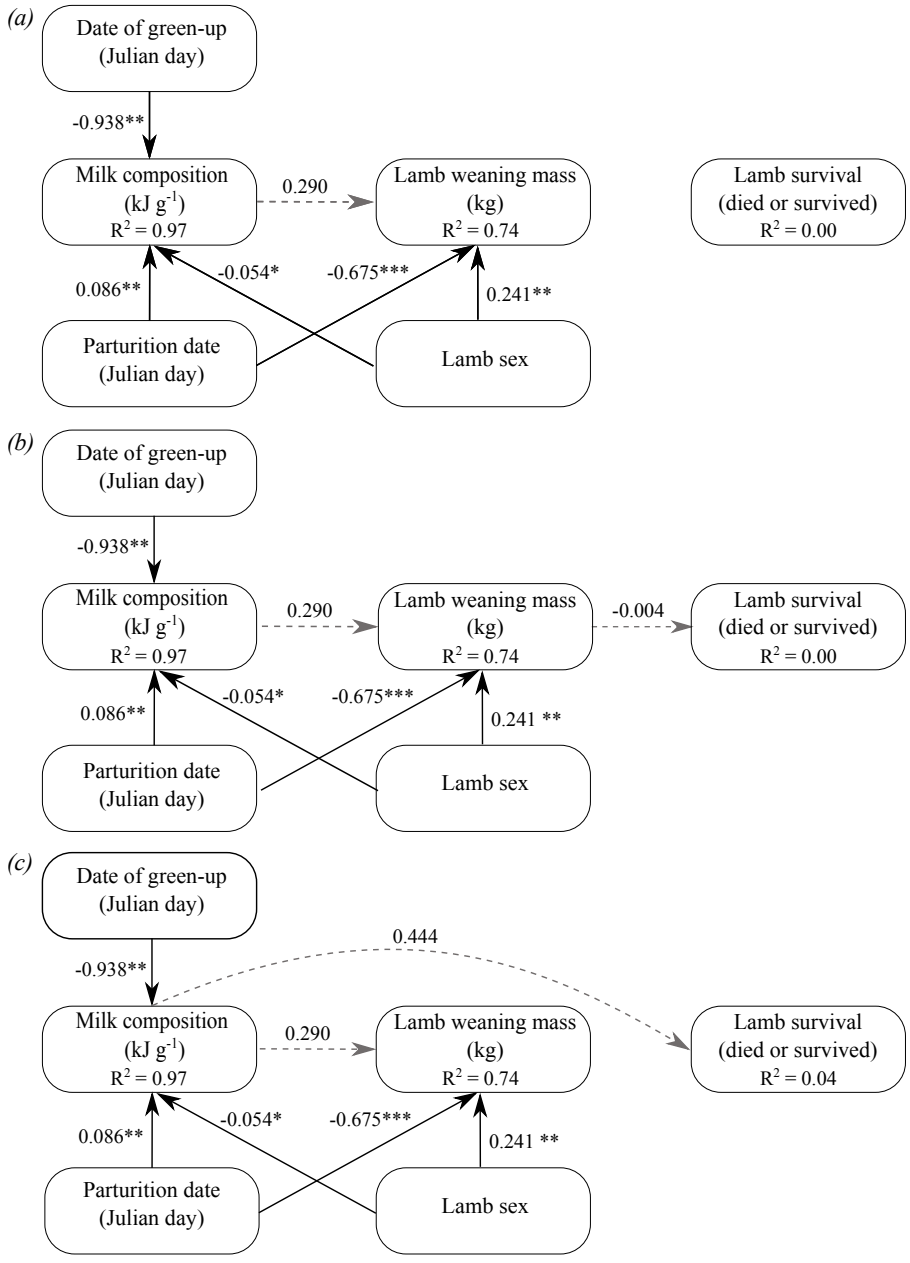


**Figure 4.6 Path diagrams of the phenological and lamb variables that drive inter-annual variation in milk composition, bighorn lamb weaning mass and overwinter survival probability. Bighorn sheep data are from Ram Mountain, Alberta, Canada, 2011 – 2016 (n = 65).**

We first tested the absence of effect on survival in *(a)*; we tested an indirect effect of milk energy on lamb survival in *(b)* and a direct effect of milk energy on lamb survival in *(c)*. Dashed arrows indicate insignificant paths included in a model; solid arrows indicate significant effects of a variable on another. Standardized path coefficients are indicated directly on corresponding paths (*** : P = 0 – 0.001, ** : P = 0.001 – 0.01, * : P = 0.01 – 0.05)

## 4.6 Discussion

Phenological changes in reproductive traits following climate change are well documented [2] but most evidence in vertebrates comes from birds [8, 52, 5, 16], especially small passerines [10]. We quantified temporal variation in spring phenology and investigated how it influenced phenotypic traits during the first year of life, given that early-life traits can have long-term fitness effects [1]. We found that neonatal survival probability was higher with earlier parturition dates. Longer vegetation growing seasons increased milk fatty acid concentrations, but we found no causal relationships between milk composition, lamb weaning mass and lamb overwinter survival. Our results nevertheless show that spring vegetation phenology influences bighorn sheep reproduction especially during lactation, and suggest that large mammals in seasonal environments also rely on local resources as a breeding strategy during lactation [11].

We found no clear pattern in neonatal survival and spring phenology over years. This lack of change is surprising given that global climate change has led to warmer and earlier spring elsewhere [53]. Our study area has however undergone declines in autumn precipitation and increases in autumn temperatures over the last 26 years [54]. Parturition dates advanced by $\sim$ 15 days, partly through within-individual phenotypic plasticity in response to autumn weather [54]. Here, we found that lambs that die neonatally are born slightly later than survivors, but distributions of parturition dates from neonatal deaths and survivors were nearly identical and both distributions overlapped green-up dates. Late parturition date, however, decreased the probability of neonatal survival. The deviation between parturition date and green-up date also decreased neonatal survival although this effect was not significant. Mothers breeding late might not benefit from fresh and plentiful vegetation early enough to provide good quality milk and maternal care. Because gestation length in bighorn sheep varies by less than 1 day [55], neonatal mortality may increase with late parturition because late-breeding ewes are in poorer condition.

Short 'snow-free' seasons, and long plant productivity seasons, were associated with high neonatal survival with a one year lag, suggesting an important role of previous summer forage on body condition [20]. For example, habitats with abundant forage are associated with increased fat reserves in white-tailed deer (*Odocoileus virginianus*) [56]. Prolonged periods of plant productivity would have improved bighorn ewe nutrition, increased their fat reserves in autumn and improved their ability to care for neonates based on body reserves the following spring. The

positive effect of short 'snow-free' seasons on neonatal survival is surprising given that snow depth limits space use, increase energy expenditure during movements and limits resource availability for alpine ungulates [57]. Perhaps long-lasting snow cover indirectly influences some species of plants through modified soil characteristics (i.e. increased soil temperature, microbial activity and shrub abundance [58]) but we could not measure these changes. Finally, parasite loads, mainly lungworms, affect maternal condition, lamb viability and nursing behaviours [39] and lungworm infections are associated with pneumonia in bighorn sheep [59]. Intermediate hosts are snails that are favoured by wet conditions [59]. Lungworms migrate to vegetation ingested by sheep thus perhaps short 'snow-free' seasons prevent snail proliferation and further migration from sheep fecal material to snail hosts. Positive effects of short 'snow-free' season on neonatal survival otherwise remain unexplained.

Following years with high predation, neonatal survival was reduced possibly due to indirect effects on maternal reproduction. Cougar predation on bighorn sheep in our study caused population declines [22]. Predation appears largely due to a specialist individual because cougars rarely kill sheep but in a few years they prey upon them very heavily [21, 22]. Based on results from another bighorn sheep population, we hypothesize that high predation events could also indirectly affect demography by lowering maternal effort during years with high predation, leading to reduced lamb size in autumn and lower survival [60]. In our study, we show that years with high predation risks lead to higher neonatal mortality in the following spring. We suggest that this effect may be due to either increased stress levels of females and/or reduced condition due to higher vigilance rate and reduced time spent foraging [23] (but see [24]).

Both long 'snow-free' and growing seasons increased milk fatty acid, iron and lactose concentrations, suggesting that abundant forage directly facilitates allocation of resources to lactation, perhaps through increased fat gain. In the Greater Yellowstone Ecosystem, migratory elk can track the green-up period and occupy habitat patches at peak forage quality for a longer period than resident elk. This behaviour can account for up to 6 kg of variation in body-fat levels in September [61]. Yellow-bellied marmots (*Marmota flaviventris*) emerge earlier from hibernation and benefit from longer growing seasons, allowing greater mass gain in young and adults and increased population growth [3]. We suggest that milk quality or quantity provided by the mothers, as females' summer nutrition was likely improved by longer plant availability, contributed to the observed mass gain in young marmots. However, studies on wild mammals usually do not have access to data on milk composition, limiting our ability to test the links

between change in spring phenology and milk composition. Here, we show that 'matching' spring conditions influence neonatal survival and that late spring green-up is associated with low milk energy content. However, milk composition does not influence lamb weaning mass and overwinter survival, contrasting with results for other mammals [30, 33]. Milk quantity, rather than milk composition, might drive lamb summer growth [62], but this information was unavailable in our study, although relative contribution of milk to solid food in the diets of individual lambs could be compared using stable isotope analyses [63]. Lambs also forage during summer thus they do not entirely depend on maternal milk for their growth, which might be different in other mammal species such as in marmots [3]. Interestingly, maternal mass or mass changes do not influence milk composition, partly supporting the greater importance of income resources relative to endogenous reserves for lactation in this species. This finding is surprising given the fitness implications of body mass in bighorn sheep [64]. However, females unable to produce lambs do not appear in the analyses, that is individuals might have been subject to viability selection before the trait is measured [65]. This might have hindered variation in milk composition or female body condition. Finally, our sample size and the lack of information on the exact date of weaning and thereof variation in weaning mass may have limited our capacity to detect possibly weak correlations between milk composition, weaning mass and survival as we only had 6 years of data on milk composition.

We used unique, individual-based data to investigate the effects of vegetation phenology at the population, individual and physiological levels in a wild mammal. We showed that vegetation phenology influences not only timing of breeding but also influences development and early-life history of young mammals through a fine-scale physiological integration in milk composition, all of which potentially having long-term fitness consequences. Our results suggest that bighorn ewes do not entirely sustain the costs of early lactation from stored energy reserves, and like several other mammals rely on a strategy intermediate between 'capital' and 'income' breeding when energy demands are high. Both indirect effect of predation and changes in phenology affected neonatal survival, an important but under-studied aspect of population dynamics. Young mammals might respond positively to phenological shifts by an increased mass gain during longer summers [3] but long term effects of warmer or earlier springs on vegetation growth and herbivores are unknown [53]. If species cannot adjust to changes in ecosystem productivity under climate change, the demographic consequences will threaten the persistence of wild populations.

## 4.7 Ethics

Animals were captured and handled in compliance with the Canadian Council on Animal Care, under the approval of the Université de Sherbrooke Animal Care Committee (protocol number FP- 2016-01 and MFB-2014-01—Université de Sherbrooke).

## 4.8 Data accessibility

The datasets analyzed during the current study are available from the corresponding author on reasonable request.

## 4.9 Author's contributions

Author Contributions statement :

- L.-A.R., F.G.B., A.A.C., M.F.B and F.P. conceived the ideas and designed methodology ;
- F.P. and M.F.B. run the long-term project ;
- L.-A.R. and F.R. extracted all phenology indices ;
- L.-A.R and F.R. analyzed the data ;
- L.-A.R. led the writing of the manuscript. All authors contributed critically to the drafts and gave final approval for publication.

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CHAPITRE 5

# DISCUSSION ET CONCLUSION GÉNÉRALE

## 5.1 Résumé des chapitres

Les changements climatiques surviennent rapidement et perturbent les séquences de la phénologie de la végétation. Les espèces animales montrent plusieurs mécanismes pour s'ajuster à ces changements, incluant la plasticité phénotypique. Or, bien qu'il y ait beaucoup d'évidences de réponses phénologiques chez les oiseaux, certains mammifères et les insectes, les évidences sont plus rares chez les espèces longévives qui ont des réserves corporelles et ne dépendent pas entièrement des ressources de nourriture à un moment précis pour leur reproduction. Les évidences d'ajustement chez les grands mammifères restent à ce jour assez minces, bien que les menaces futures des changements climatiques soient très présentes.

Grace à un suivi détaillé à long terme, ma thèse a pu montrer, dans un premier temps, que la plasticité phénotypique est un mécanisme utilisé par le mouflon d'Amérique pour répondre aux changements rapides de son environnement (chapitre 2). L'aire d'étude n'échappe pas au réchauffement, qui résulte principalement en une augmentation des températures automnales de 2,9°C, et une diminution des précipitations de 0,7 mm/jour à l'automne en 26 ans d'étude. En réponse à ces variations, les dates de mise-bas chez le mouflon sont avancées de 15,7 jours. Mes résultats montrent que, contrairement à plusieurs espèces d'oiseaux, les mouflons répondent aux conditions automnales et non printanières. Ceci s'explique principalement par une histoire de vie différente ; alors que beaucoup d'espèces d'oiseaux, comme les passereaux, se reproduisent principalement sur la base de ressources disponibles au printemps, les grands mammifères alpins peuvent utiliser une partie de leurs réserves de graisses pour pallier les coûts de la reproduction. Or, chez le mouflon, ces réserves de graisses s'accumulent principalement à l'été et l'automne qui précèdent les mise-bas, et elles déterminent en grande partie la masse des femelles au moment de la conception, à la fin de l'automne. Ce sont donc les facteurs limitant le moment de la conception qui déterminent la flexibilité dans les dates de mise-bas, puisque la longueur de la gestation est fixe chez cette espèce (Hogg et al., 2017). Ce

chapitre met en lumière l'importance de l'échelle temporelle dans l'étude de la plasticité phénotypique, puisque les espèces longévives ne sont pas toutes flexibles au même stade de leur reproduction. Mes analyses suggèrent que les individus s'ajustent par plasticité individuelle de façon similaire les uns aux autres, ce qui explique la réponse observée à l'échelle populationnelle. Ce travail démontre que l'environnement affecte la population et l'individu. Il est donc logique de soupçonner qu'il influence aussi la physiologie. Pour examiner cette possibilité, je me suis intéressée à un aspect physiologique des soins maternels, la composition du lait, dans le chapitre 3.

Suivant la mise-bas, le mouflon d'Amérique donne des soins maternels prolongés sur une période qui s'étend sur près d'une demi-année ; cette période est principalement caractérisée par la lactation. Nous avons montré que les variations interannuelles de l'environnement ont une incidence directe sur la composition du lait, qui varie très peu entre les femelles (chapitre 3). Ces résultats illustrent que les différentes échelles spatiales et temporelles sont très importantes à considérer dans l'interprétation de processus écologiques. Par une nouvelle approche méthodologique, nous avons abordé une problématique qui touche une majorité d'études en écologie : une grande quantité de traits est souvent mesurée sur un nombre limité d'individus. Cette problématique complique la tâche aux écologistes qui tentent de relier les traits physiologiques ou comportementaux aux mesures de valeur adaptative comme la survie et la reproduction. D'une part, les multiples traits mesurés varient entre eux et à travers le temps et les stades d'histoire de vie des individus. D'autre part, le faible nombre d'observations combiné au grand nombre de traits rend presque impossible la tâche d'élucider les (co)variances entre les traits. C'est pourquoi nous suggérons une approche par modèle mixte multivarié, avec variables latentes, pour aborder la complexité liée aux traits d'histoire de vie. Les variables latentes réduisent le nombre de « dimensions » à estimer pour étudier les corrélations entre les traits. Leur utilisation facilite grandement la décomposition de la matrice de (co)variances tant aux échelles individuelles qu'annuelles. Ces analyses ont été déterminantes pour le chapitre 4, puisque la grande variation environnementale observée dans la composition du lait demeurait à ce moment inexpliquée. Chez un herbivore, il semblait logique de s'interroger sur la phénologie du printemps et ses effets sur des traits liés aux soins maternels.

Dans mon quatrième chapitre, je me suis intéressée à un paradigme à l'origine d'une hypothèse omniprésente en écologie : celle qui suppose que le moment de la mise-bas est optimal lorsqu'il coïncide avec le pic d'abondance de nourriture au printemps. Le raisonnement derrière cette

hypothèse vient du fait que dans les milieux saisonniers, la croissance des jeunes en été est déterminante pour leur survie au premier hiver. Cette croissance dépend donc de la qualité ou de la quantité de nourriture disponible au printemps et en été. Sachant que les dates de mise-bas ne sont pas déterminées par la phénologie du printemps (chapitre 2), j'ai voulu vérifier si la survie néonatale, la composition du lait et la masse au sevrage étaient influencées par les conditions printanières, incluant la date de la mise-bas. En utilisant des indices de végétation dérivés d'images satellitaires, j'ai montré que des variables clés dans les stades de vie qui suivent les mise-bas répondent aux variations en productivité primaire et en couvert de neige au sol. Les agneaux qui survivent au stade néonatal sont nés plus tôt que ceux qui meurent à ce stade ; surtout, les « survivants » du stade néonatal sont nés quelques jours avant la date d'émergence de la végétation, ce qui donne possiblement une longueur d'avance aux mères pour profiter de cette nourriture fraîche et prodiguer les soins parentaux par la suite. Nous avons aussi montré qu'une forte prédation augmente les mortalités néonatales au printemps suivant, ce qui suggère un effet indirect de la prédation sur le recrutement. Une longue saison de productivité primaire, durant l'été précédant les naissances, améliore la survie néonatale au printemps suivant. Une longue saison de productivité primaire enrichit aussi la composition du lait ; les mères qui ont accès à des ressources de meilleure qualité pour une période plus longue et sans neige ont des laits plus riches en acides gras, sucre et minéraux. Une longue période sans neige influence positivement la composition du lait, mais réduit la survie néonatale au printemps suivant. Ce chapitre teste donc directement l'idée que donner naissance en synchronie avec la disponibilité de nourriture peut influencer les traits liés aux soins maternels.

Dans leur ensemble, mes travaux démontrent l'importance de se baser sur de longues séries temporelles climatiques et phénologiques pour bien comprendre les causes et les conséquences de la variation phénotypique chez les organismes vivants. Mes travaux mettent l'emphase sur l'importance des suivis de populations à long terme, qui permettent de documenter les impacts de la variabilité environnementale qu'un individu expérimentera au cours de sa vie. Finalement, mon travail souligne la nécessité d'utiliser les outils appropriés pour aborder ces questions complexes.

## 5.2 Limites et perspectives de l'étude

### 5.2.1 Réponses phénologiques dans un environnement changeant

Dans le chapitre 2, l'objectif était de comprendre comment les variations de l'environnement affectent la réponse populationnelle et individuelle en dates de mise-bas. Nous avons principalement montré une réponse face aux précipitations et températures, mais l'effet de la température disparaissait lorsque la variable était dissociée de sa tendance temporelle. Plusieurs raisons peuvent expliquer ces résultats. D'abord, nos modèles de plasticité étaient conservateurs car ils incluaient l'année et empêchaient probablement de détecter les effets liés aux variables environnementales, si ces effets étaient faibles. Ensuite, il est possible que les indices météorologiques et climatiques, comme la température et un indice climatique, le PDO, soient trop indirectement liés aux facteurs importants pour l'écologie du mouflon pour engendrer une réponse phénotypique. Dans notre aire d'étude, il n'y a aucun suivi de végétation et les données de phénologie extraits d'images satellitaires (chapitre 4) n'étaient pas disponibles jusqu'à récemment. Par conséquent, nous avons dû utiliser les indices indirects disponibles à ce moment, mais il est possible qu'ils ne soient pas fiables ni informatifs sur la prévisibilité de l'environnement (Bonamour et al., 2019). Il serait donc intéressant, à l'avenir, d'intégrer les indices de phénologie à l'automne pour tester comment la croissance (ou sénescence) de la végétation affecte la plasticité en dates de conception et de mise-bas. Ceci permettrait aussi de mieux comprendre l'aspect multidimensionnel de la plasticité dans les environnements complexes (Bonamour et al., 2019). De même, les événements climatiques extrêmes pourraient être de plus en plus importants dans le futur, et nous ne connaissons presque rien de leurs conséquences écologiques et évolutives (e.g., Marrot et al., 2017).

L'absence de réponse plastique aux variables autres que les précipitations peut être expliquée par des contraintes à la plasticité, notamment les conditions à la naissance des mères (Nussey et al., 2005) ou encore, parce qu'une réponse aurait été maladaptative (Ghalambor et al., 2007). Une densité élevée à la naissance peut, en diminuant les ressources disponibles par individu, avoir un effet important à long-terme sur la valeur adaptative des individus adultes (Pigeon et Pelletier, 2018) et restreindre leur capacité à répondre aux conditions environnementales à l'âge adulte. Une forte densité à l'âge adulte est aussi synonyme de diminution des ressources *per capita*. Les dates de mise-bas sont récoltées à Ram Mountain depuis 1992, soit la période

à partir de laquelle la densité de population s'est mise à diminuer radicalement et de façon presque continue (Figure 5.1). Même en dissociant cette variable de sa tendance temporelle, nous n'avons pas trouvé d'effets de densité sur les dates de mise-bas, bien que ce soit un déterminant dans la phénologie de reproduction d'autres mammifères (Cordes et Thompson, 2013). Nous n'avons pas non-plus détecté de variations en normes de réaction, c'est-à-dire que l'interaction « individu-par-environnement » n'était pas significative pour aucune des variables testées. Nous avons voulu vérifier si l'influence de la densité à la naissance des mères réprimait la variation dans leurs dates de mise-bas. Il nous a été impossible de détecter un effet, mais il serait intéressant de tester si d'autres facteurs physiologiques (e.g., hormonaux, génétiques) empêchent les femelles de varier dans leur réponse plastique. Une avenue potentielle serait d'étudier comment les facteurs démographiques autres que la densité de population, par exemple la structure d'âge (Figure 5.1), influencent les dates de mise-bas (Chevin et al., 2013). D'un autre côté, l'absence de changement en date de mise-bas en réponse à l'augmentation de densité peut indiquer qu'un changement aurait peu d'avantages en termes de valeur sélective, si on considère que les ressources seraient peu disponibles pour les nouveau-nés et leur mères.

Il avait été précédemment montré que les dates de mise-bas avaient une influence sur la survie des agneaux (Feder et al., 2008) ainsi que chez d'autres ongulés (Plard et al., 2015). D'une part, une perspective intéressante serait de bonifier ces travaux en effectuant une analyse de sélection sur les dates de mise-bas. Cette analyse confirmerait si les dates de mise-bas sont sous sélection directionnelle (si mettre bas de plus en plus tôt augmente la valeur adaptative, il devrait y avoir une relation linéaire avec le *fitness* relatif des individus) ou stabilisante (si les dates de mises-bas sont optimales à une période spécifique, par exemple à la date moyenne d'émergence de la végétation, il devrait y avoir une relation quadratique avec le *fitness* relatif). D'autre part, il est connu que les changements phénotypiques liés à la phénologie influencent la dynamique de population (Ozgul et al., 2010). Il serait donc pertinent d'intégrer directement la variation phénotypique en dates de mise-bas dans un modèle démographique (Pelletier et al., 2007) afin de connaître l'étendue des valeurs du phénotype (ici date de mise-bas) qui contribue à un taux de croissance positif de la population.

Parmi ses limites connues (Auld et al., 2010), la plasticité n'agit qu'à court terme. Des réponses à plus long terme seront nécessaires pour éviter l'extinction en présence de changements de plus en plus importants. Ces réponses à long terme impliquent notamment des changements génétiques. Nous savons que la date de mise-bas est un trait héritable ($h^2 = 0.13$ chez le mou-

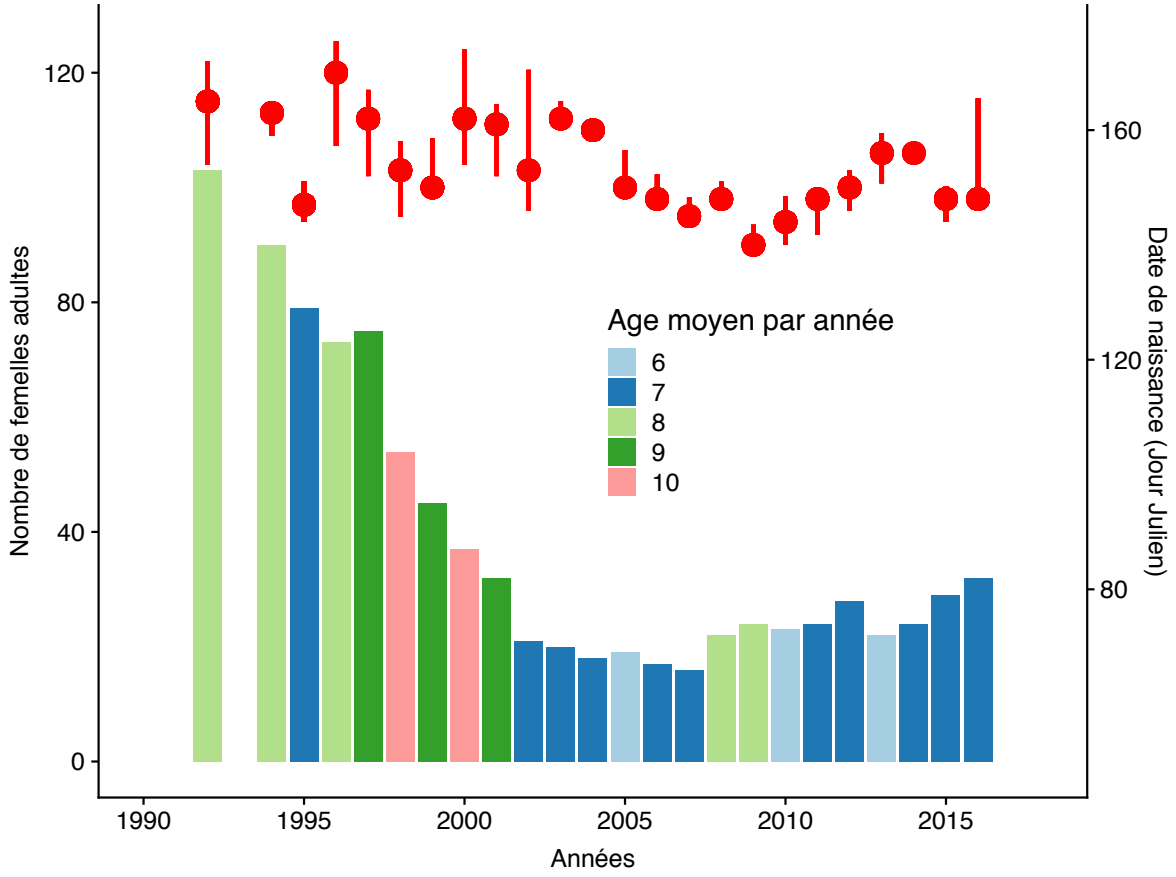


**Figure 5.1 Densité de population, structure d'âge des femelles adultes et dates de mise-bas des mouflons d'Amérique à Ram Mountain, Alberta, Canada, 1992-2016.**

La structure d'âge représente la moyenne d'âge des femelles adultes par année. La date de mise-bas médiane, le premier et le troisième quartiles sont illustrés par année. Les dates de mise-bas ne sont pas disponibles en 1993.

flon, Feder et al., 2008). Il serait peut-être pertinent d'analyser si les changements en dates de mise-bas peuvent aussi provenir d'une réponse microévolutive. Même si la contribution relative d'un changement génotypique risque d'être plus faible que celle d'un changement phénotypique (Gienapp et al., 2008 ; Pigeon et al., 2017), les réponses évolutives peuvent survenir rapidement (Ellner, 2013) et tant les réponses plastiques qu'évolutives peuvent influencer la persistance d'une population (Chevin et al., 2010). Modéliser la contribution relative de ces deux mécanismes sur la trajectoire de population, en incluant les seuils de tolérance face aux variables climatiques et météorologiques locales, serait donc extrêmement intéressant. Toutefois, la petite taille d'échantillon, le choix des variables environnementales, la grande part de variation inexpliquée (du fait des variables environnementales choisies) et potentiellement l'incertitude autour des estimés de dates de naissance limitaient la puissance de nos analyses de plasticité. Si la population continue d'augmenter à Ram Mountain dans les prochaines années, la taille d'échantillon pourrait être bonifiée ce qui serait nécessaire pour réaliser ce projet.

### 5.2.2 Les approches multitraits

Pour mieux prédire la trajectoire de population et les changements en valeur adaptative, il est suggéré de modéliser plusieurs traits simultanément (Chevin et al., 2013). En effet, les traits sont souvent considérés comme indépendants les uns des autres, mais cette supposition est rarement vraie (Dochtermann et Jenkins, 2007 ; Araya-Ajoy et Dingemanse, 2013). Dans un contexte de changements globaux, une approche multitrait pourrait apporter des éléments de compréhension nouveaux et intégrateurs. Même si ce sont les dates de mise-bas ou d'émergence de l'hibernation qui coïncident ou non avec l'abondance des ressources, nous savons aussi que plusieurs traits répondent à la phénologie (chapitre 4). Notamment, il y a très peu d'information sur la survie néonatale en milieu naturel, à savoir si ce trait est adaptatif ou non. Une approche multitrait permettrait de saisir toute l'information contenue dans les (co)variances entre les traits liés à la phénologie, et de comprendre si certaines corrélations négatives empêchent l'ajustement aux changements (Walling et al., 2014). L'approche multitrait devrait être préconisée lorsque la corrélation entre plusieurs traits, qu'ils soient physiologiques, comportementaux ou morphologiques, est une information importante en soi (par exemple les syndromes comportementaux) et qu'elle influence la valeur adaptative des individus.

En écologie évolutive, il existe des modèles « trait-état » (‘*character-state models*’) qui re-

présentent des « modules » de traits reliés par une même fonction biologique (Houslay et al., 2019). Dans ces modèles, les valeurs d'un trait dans différents environnements sont considérées comme des trait distincts et corrélés. Nous avons montré dans le chapitre 3 qu'il existe des méthodes pour réduire le nombre de dimensions d'une matrice de (co)variances, dont les modèles hiérarchiques multivariés avec variables latentes. Avec ces méthodes, tous les paramètres de la matrice n'ont pas à être estimés à proprement parler, puisqu'un nombre réduit de variables latentes peut être utilisé, semblable aux axes d'une analyse en composante principale. Toutefois, contrairement à cette dernière analyse, nous ne cherchons pas à interpréter les variables latentes ; nous ne les utilisons que pour résoudre la matrice de (co)variances.

On constate que certains domaines de l'écologie ne sont pas enclins à utiliser de telles méthodes multivariées. Dans le cas de l'écophysiologie, par exemple, ceci est surprenant puisqu'un grand nombre de marqueurs physiologiques est nécessaire pour bien décrire la variation entre individus (Buehler et al., 2011 ; Cohen et al., 2012 ; Milot et al., 2014). En contraste, les domaines de l'écologie des communautés et de la génétique quantitative utilisent des méthodes similaires depuis plus d'une dizaine d'années déjà (Kirkpatrick et Meyer, 2004). Les études de plasticité phénotypique et les études écophysiologiques bénéficieraient d'une méthode comme celle que nous avons présentée au chapitre 3. Dans notre contexte, il aurait été intéressant de modéliser plusieurs traits liés au développement des agneaux, par exemple, les dates de naissance, la croissance, la survie néonatale, la masse à la naissance et au sevrage en fonction de plusieurs variables de phénologie ; nous aurions ainsi obtenu, en un coup d'oeil, les corrélations entre les variables de développement, et leurs réponses conjointes aux variations de l'environnement. Plus intéressant encore, nous pourrions analyser ces réponses aux échelles biologiques d'organisation qui nous intéressent, soit individuelle ou populationnelle, et même génétique si le jeu de données le permettait. Ceci étant dit, une limite du chapitre méthodologique sur la composition du lait est de n'avoir évalué que la répétabilité des composantes, et non leur plasticité. L'idée initiale était basée sur la littérature sur les soins maternels qui montrait des différences individuelles, incluant génétiques, en composition du lait (Carta et al., 2008 ; Lang et al., 2005 ; 2009). Toutefois, la littérature sur la physiologie montre clairement que les traits physiologiques peuvent être plastiques (Buehler et al., 2012). La plasticité aurait pu être testée différemment que dans le chapitre 2 puisque nous n'avions pas beaucoup d'années de captures et d'échantillons par femelle. Ceci aurait pu confirmer que plasticité et répétabilité en soins parentaux ne sont pas incompatibles (Westneat et al., 2011).

Malgré une approche par modèles hiérarchiques multivariés (chapitre 3) ou mécanistique (chapitre 4), nous n'avons pas trouvé de conséquences de la variation en composition du lait sur la survie au sevrage ou à un an. Ceci suggère que d'autres paramètres liés aux soins maternels, peut-être la quantité de lait ou d'autres soins prodigués par la mère (Théoret-Gosselin et al., 2015), seraient de meilleurs déterminants de la survie que la composition du lait. Un aspect inconnu dans plusieurs études en milieu naturel, dont la nôtre, est la quantité de lait produite par les mères. La quantité de lait peut difficilement être estimée (Cameron, 1998 ; Théoret-Gosselin et al., 2015). La diminution de la durée, du succès et de la fréquence des tétées (Berger, 1979 ; Festa-Bianchet, 1988b) est fréquemment rapportée pour illustrer la diminution de l'apport d'énergie investie par la mère dans sa progéniture à l'approche de la période de sevrage (mais voir Therrien et al. (2008) ; Théoret-Gosselin et al. (2015)). Dans notre étude, nous supposons que le sevrage survient vers la mi-septembre mais il pourrait se prolonger jusqu'au mois d'octobre (Festa-Bianchet, 1988b), puisque des différences individuelles peuvent avoir lieu aussi à travers ce processus (voir l'encadré 2 en Introduction). Les changements globaux peuvent entraîner un changement dans les dates d'émergence de la végétation, ou encore, de fonte des neiges, qui à leur tour entraînent un prolongement de la période de croissance des jeunes mammifères (Ozgul et al., 2010). Or, un prolongement de la période de productivité primaire pourrait aussi bénéficier les mères qui, profitant de ressources additionnelles, pourraient prolonger la période de lactation. Il serait donc intéressant de vérifier la présence de différences individuelles dans le moment du sevrage, afin d'en évaluer les conséquences.

Les données comportementales de notre population, ou les observations du sevrage dans une autre population, permettraient d'estimer la date du sevrage et donc la durée de la lactation. Ceci nous confirmerait qu'avec les changements globaux, tant les périodes de croissance de la végétation, de composition du lait et de croissance des jeunes mammifères sont affectées de façon simultanée. Récemment, une méthode très prometteuse a été développée chez les primates, pour évaluer à quel moment se fait la transition entre l'allaitement et l'alimentation « indépendante » (Bădescu et al., 2016). Cette méthode se base sur les changements en ratios d'isotopes stables fécaux au cours d'une saison (ou de plusieurs années, voir la figure 1.2 en Introduction), et renseigne sur l'importance relative du lait maternel et d'autres sources de nourriture dans le régime alimentaire des jeunes, et donc, sur les effets de s'alimenter à différents « niveaux trophiques » (Bădescu et al., 2016). Cette transition entre niveaux trophiques indique le sevrage, qui, avec les dates de naissance, indiquerait si la durée de la lactation varie d'une année à l'autre, ou d'un individu à l'autre. Ceci serait d'autant plus important à étudier

à long terme, car rien n'indique que les saisons de productivité primaire se décaleront éternellement pour bénéficier les organismes qui en dépendent (Buermann et al., 2018). Notamment, si les précipitations tendent à diminuer (chapitre 2), il est fort possible que certaines espèces de plantes atteignent une limite physiologique (le manque d'eau) à émerger plus tôt et à croître plus longtemps.

À ce sujet, la quantité d'information qu'on peut extraire d'images satellitaires est énorme. Nous n'avons extrait qu'une partie des paramètres des séries temporelles disponibles, et n'avons analysé qu'une partie des paramètres extraits. Il sera intéressant de se pencher un peu plus sur la signification de changements subis au niveau de la productivité primaire. Dans notre système, nous montrons que lorsque l'émergence de la végétation est hâtive (entre le 10 avril et le 20 mai), les dates de mise-bas surviennent après cette date de disponibilité de nourriture. Toutefois, un « pic » de nourriture qui survient un peu plus tard (entre le 20 mai et 29 juin) coïncide avec des naissances un peu plus hâtives, comme illustré par des déviations négatives des naissances par rapport aux dates d'émergence de la végétation (Figure 5.2). En d'autres termes, mieux quantifier l'ampleur des variations phénologiques entre les différents niveaux trophiques est important, car cette information est à la base de la notion de « décalage » (*mismatch*) qui survient lorsque la réponse phénologique à un niveau trophique est insuffisante (i.e. trop lente, trop faible) pour permettre son ajustement aux variations du niveau trophique inférieur (Both et al., 2009).

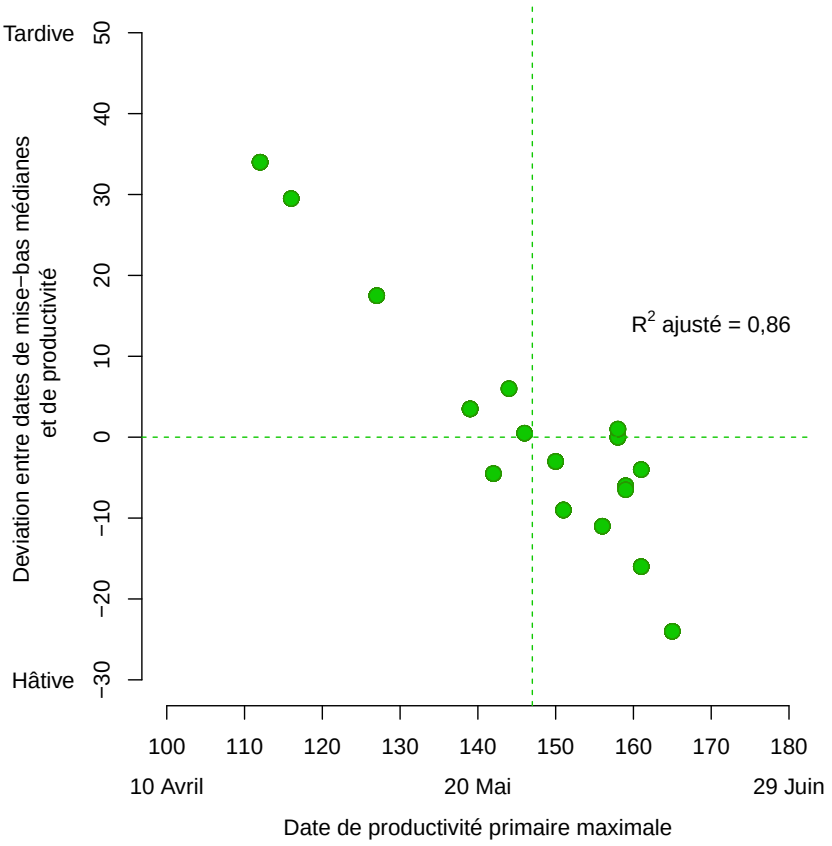


**Figure 5.2 Déviation entre les dates de mise-bas médianes des mouflons d'Amérique et les dates annuelles d'émergence de la végétation, en fonction de la date d'émergence à Ram Mountain, Alberta, Canada, 2000-2016.**

La date de mise-bas médiane et la date d'émergence moyenne pour l'ensemble des années sont indiquées au moyen d'une ligne pointillée. Les dates d'émergence sont extraites d'images satellitaires de la productivité primaire.

## 5.3 Conclusion

Cette thèse avait pour but de mieux comprendre comment les variations de l'environnement affectent la phénologie de la reproduction d'un grand herbivore terrestre. Les impacts de changements météorologiques ont été mis en évidence dans le chapitre 2, qui montrait la plasticité phénotypique en dates de mise-bas. Le chapitre 4 montrait quant à lui que les périodes de disponibilité de la végétation influencent les traits liés au développement des agneaux. Ces résultats suggèrent que les espèces capables de répondre et de s'ajuster à court terme aux changements globaux auront de meilleures chances de persister. Il sera donc intéressant de quantifier la réponse à plus long terme des espèces, par exemple par microévolution, et les réponses démographiques des populations, en réponse aux perturbations du climat. Le chapitre 3 a permis d'illustrer la nature multidimensionnelle des traits d'histoire de vie, en suggérant une méthode pour aborder cette complexité. Ce chapitre se voulait un exemple d'application aux données écophysiologiques, qui reflètent bien la problématique de mesurer de multiples traits sur un nombre restreint d'individus en milieu naturel. En conclusion, cette thèse illustre une infime partie des conséquences des changements climatiques sur la biodiversité. Les projections des impacts futurs des changements globaux seront facilitées par une meilleure compréhension des réponses écologiques, comportementales et évolutives face aux expressions complexes du changement climatique et des évènements météorologiques et climatiques extrêmes.

ANNEXE A

# ANNEXE CHAPITRE 2

## Phenotypic plasticity in bighorn sheep reproductive phenology : from individual to population

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**Table A.1 Environmental variables included in the analyses of environmental determinants prior to standardization.**

| Environmental variable | Range | Mean | Standard deviation |
|---|---|---|---|
| Precipitation (mm/day) | 0.07 – 2.10 | 0.74 | 0.51 |
| Temperature (°C) | 0.49 - 5.34 | 3.75 | 1.14 |
| Adult female density | 16 – 97 | 40.72 | 27.34 |
| Summer PDO | -1.80 - 2.57 | 0.06 | 1.20 |

**Table A.2 Determinants of parturition dates in bighorn sheep at the population level (n = 394 dates), 1992 to 2017, Ram Mountain, Alberta, Canada.**

| Fixed effects | Estimates | 95% $CI_{Lower}$ | 95% $CI_{Upper}$ | Random effects | Variance |
|---|---|---|---|---|---|
| a) Autumn precipitation | | | | | |
| Intercept (no lamb) | 2.77 | 2.59 | 2.96 | Female identity | 0.03 |
| Autumn precipitation | 0.27 | 0.11 | 0.43 | Year | 0.11 |
| Not weaned | -0.13 | -0.30 | 0.04 | Residual | 0.24 |
| Weaned | 0.08 | -0.08 | 0.24 | $R^2_{marginal}$ | 0.20 |
| Maternal mass | -0.10 | -0.16 | -0.03 | $R^2_{conditional}$ | 0.49 |
| b) Autumn temperature | | | | | |
| Intercept (no lamb) | 2.82 | 2.62 | 3.02 | Female identity | 0.03 |
| Autumn temperature | -0.13 | -0.28 | 0.02 | Year | 0.16 |
| Not weaned | -0.14 | -0.30 | 0.04 | Residual | 0.24 |
| Weaned | 0.07 | -0.09 | 0.24 | $R^2_{marginal}$ | 0.08 |
| Maternal mass | -0.10 | -0.17 | -0.03 | $R^2_{conditional}$ | 0.47 |
| c) Adult female density | | | | | |
| Intercept (no lamb) | 2.81 | 2.61 | 3.02 | Female identity | 0.03 |
| Female density | -0.14 | -0.31 | 0.04 | Year | 0.15 |
| Not weaned | -0.14 | -0.31 | 0.03 | Residual | 0.24 |
| Weaned | 0.07 | -0.09 | 0.24 | $R^2_{marginal}$ | 0.08 |
| Maternal mass | -0.09 | -0.16 | -0.03 | $R^2_{conditional}$ | 0.48 |
| d) Summer PDO | | | | | |
| Intercept (no lamb) | 2.83 | 2.61 | 3.04 | Female identity | 0.03 |
| Summer PDO | 0.01 | -0.16 | 0.19 | Year | 0.17 |
| Not weaned | -0.14 | -0.31 | 0.03 | Residual | 0.24 |
| Weaned | 0.07 | -0.09 | 0.24 | $R^2_{marginal}$ | 0.04 |
| Maternal mass | -0.09 | -0.16 | -0.03 | $R^2_{conditional}$ | 0.48 |

Estimates of 'detrended' variables and their 95% confidence intervals are reported at the population level. Female identity and year were included as random intercepts in all models. Marginal and conditional $R^2$ for fixed and random effects are indicated below random effects.

**Table A.3 Random regression analyses to assess variation in plasticity, i.e. an individual-by-environment ($I \times E$) component, in bighorn sheep parturition dates (n = 350), 1992 to 2017, Ram Mountain, Alberta, Canada.**

| Precipitation | Log-likelihood | Test | df | LRT | P-value |
|---|---|---|---|---|---|
| 1. Year | -292.48 | - | 8.00 | - | - |
| 2. Year + Female | -288.72 | 1 vs 2 | 9.00 | 7.53 | 0.006 |
| 3. Year + Female x Precipitation$_{within}$ | -287.93 | 2 vs 3 | 11.00 | 1.57 | 0.46 |
| Temperature | | | | | |
| 4. Year | -294.73 | - | 8.00 | - | - |
| 5. Year + Female | -291.58 | 4 vs 5 | 9.00 | 6.30 | 0.01 |
| 6. Year + Female x Temperature$_{within}$ | -290.56 | 5 vs 6 | 11.00 | 2.03 | 0.36 |
| Density | | | | | |
| 7. Year | -295.71 | - | 8.00 | - | - |
| 8. Year + Female | -292.33 | 7 vs 8 | 9.00 | 6.76 | 0.009 |
| 9. Year + Female x Density$_{within}$ | -290.79 | 8 vs 9 | 11.00 | 3.07 | 0.22 |
| Summer PDO | | | | | |
| 10. Year | -296.69 | - | 8.00 | - | - |
| 11. Year + Female | -293.08 | 10 vs 11 | 9.00 | 7.22 | 0.007 |
| 12. Year + Female x PDO$_{within}$ | -292.36 | 11 vs 12 | 11.00 | 1.44 | 0.49 |

Results were obtained after all explicative variables were 'detrended' following Grosbois et al. (2008) but their equivalent models on initial predictors can be found in the main text. Three models with an increasingly complex random structure were compared for each of the four environmental variables : autumn precipitation, temperature, adult female density and summer PDO. Random structures of models 1 to 3, 4 to 6, 7 to 9 and 10 to 12 were compared with a LTR. All models included the within-individual ($\beta_W$) and the between-individual ($\beta_B$) components of the environmental variable as fixed predictors. All models included as control variables maternal mass in September and previous reproductive success (a 3-level factor). Within-individual centering was applied as suggested by van de Pol and Wright (2009)

**Table A.4 Parameter estimates of linear mixed-effects models of the effects of between-individual ($\beta_B$) and within-individual ($\beta_W$) components of a) precipitation, b) temperature, c) adult female density and d) summer PDO on 350 bighorn sheep parturition dates, 1992 to 2017, Ram Mountain, Alberta, Canada.**

| Fixed effects | Estimate | 95% CI | Random effects | Variance |
|---|---|---|---|---|
| a) Autumn precipitation | | | | |
| Intercept (no lamb) ($\beta_0$) | 2.79 | 2.58 – 3.00 | Female (Intercept) | 0.03 |
| Maternal mass | -0.09 | -0.16 – -0.02 | Year (Intercept) | 0.11 |
| PRS : not weaned | -0.18 | -0.36 – 0.02 | Residual | 0.24 |
| PRS : weaned | 0.05 | -0.13 – 0.23 | $R^2_{marginal}$ | 0.18 |
| Precipitation$_{between}(\beta_B)$ | 0.28 | 0.07 – 0.49 | $R^2_{conditional}$ | 0.48 |
| Precipitation$_{within}(\beta_W)$ | 0.25 | 0.10 – 0.40 | | |
| b) Autumn temperature | | | | |
| Intercept (no lamb) ($\beta_0$) | 2.83 | 2.61 – 3.06 | Female (Intercept) | 0.03 |
| Maternal mass | -0.09 | -0.16 – -0.02 | Year (Intercept) | 0.15 |
| PRS : not weaned | -0.18 | -0.37 – 0.01 | Residual | 0.25 |
| PRS : weaned | 0.05 | -0.13 – 0.23 | $R^2_{marginal}$ | 0.08 |
| Temperature$_{between}(\beta_B)$ | -0.20 | -0.39 – 0.00 | $R^2_{conditional}$ | 0.47 |
| Temperature$_{within}(\beta_W)$ | -0.12 | -0.27 – 0.03 | | |
| c) Female density | | | | |
| Intercept (no lamb) ($\beta_0$) | 2.83 | 2.60 - 3.05 | Female (Intercept) | 0.03 |
| Maternal mass | -0.09 | -0.16 - -0.02 | Year (Intercept) | 0.16 |
| PRS : not weaned | -0.18 | -0.37 - 0.01 | Residual | 0.25 |
| PRS : weaned | 0.04 | -0.14 - 0.22 | $R^2_{marginal}$ | 0.08 |
| Density$_{between}(\beta_B)$ | -0.15 | -0.35 - 0.04 | $R^2_{conditional}$ | 0.48 |
| Density$_{within}(\beta_W)$ | -0.12 | -0.29 - 0.05 | | |
| d) Summer PDO | | | | |
| Intercept (no lamb) ($\beta_0$) | 2.85 | 2.62 - 3.07 | Female (Intercept) | 0.03 |
| Maternal mass | -0.09 | -0.16 - -0.02 | Year (Intercept) | 0.17 |
| PRS : not weaned | -0.19 | -0.38 - 0.01 | Residual | 0.25 |
| PRS : weaned | 0.04 | -0.14 - 0.22 | $R^2_{marginal}$ | 0.04 |
| PDO$_{between}(\beta_B)$ | -0.04 | -0.26 - 0.17 | $R^2_{conditional}$ | 0.47 |
| PDO$_{within}(\beta_W)$ | 0.03 | -0.14 - 0.21 | | |

Results were obtained after all explicative variables were 'detrended' following Grosbois et al. (2008). Estimates of fixed effects and variance components of random effects of models 2, 5, 8 and 11 are presented based on results from random regression analyses and LRT in Table A.3. Within-individual centering was applied as suggested by van de Pol and Wright (2009). PRS : reproductive success the previous year. Estimates of models on initial predictors (not detrended) are in the main text.

**Table A.5 Parameter estimates of random regressions of the effects of between-individual ($\beta_B$), within-individual ($\beta_W$) components and $I \times E$ in response to a) precipitation, b) temperature, c) adult female density and d) summer PDO on 350 bighorn sheep parturition dates, 1992 to 2017, Ram Mountain, Alberta, Canada.**

| Fixed effects | Estimate | 95% CI | Random effects | Variance |
|---|---|---|---|---|
| a) Autumn precipitation | | | | |
| Intercept (no lamb) ($\beta_0$) | 2.81 | 2.62 - 3.00 | Female (Intercept) | 0.03 |
| Maternal mass | -0.09 | -0.16 - -0.02 | I X E | 0.002 |
| PRS : not weaned | -0.18 | -0.37 - 0.01 | Year (Intercept) | 0.06 |
| PRS : weaned | 0.03 | -0.15 - 0.21 | Residual | 0.24 |
| Precipitation$_{between}(\beta_B)$ | 0.42 | 0.25 - 0.59 | $R^2_{marginal}$ | 0.26 |
| Precipitation$_{within}(\beta_W)$ | 0.28 | 0.16 - 0.40 | $R^2_{conditional}$ | 0.46 |
| b) Autumn temperature | | | | |
| Intercept (no lamb) ($\beta_0$) | 2.88 | 2.68 - 3.07 | Female (Intercept) | 0.03 |
| Maternal mass | -0.09 | -0.16 - -0.02 | I X E | 0.01 |
| PRS : not weaned | -0.22 | -0.40 - -0.04 | Year (Intercept) | 0.09 |
| PRS : weaned | 0.04 | -0.17 - 0.19 | Residual | 0.24 |
| Temperature$_{between}(\beta_B)$ | -0.34 | -0.49 - -0.19 | $R^2_{marginal}$ | 0.21 |
| Temperature$_{within}(\beta_W)$ | -0.17 | -0.34 - -0.01 | $R^2_{conditional}$ | 0.48 |
| c) Female density | | | | |
| Intercept (no lamb) ($\beta_0$) | 2.88 | 2.66 - 3.09 | Female (Intercept) | 0.03 |
| Maternal mass | -0.09 | -0.17 - -0.03 | I X E | 0.001 |
| PRS : not weaned | -0.20 | -0.39 - -0.01 | Year (Intercept) | 0.13 |
| PRS : weaned | 0.02 | -0.16 - 0.20 | Residual | 0.24 |
| Density$_{between}(\beta_B)$ | 0.21 | 0.05 - 0.37 | $R^2_{marginal}$ | 0.11 |
| Density$_{within}(\beta_W)$ | 0.04 | -0.18 - 0.26 | $R^2_{conditional}$ | 0.46 |
| d) Summer PDO | | | | |
| Intercept (no lamb) ($\beta_0$) | 2.86 | 2.63 - 3.08 | Female (Intercept) | 0.03 |
| Maternal mass | -0.07 | -0.14 - 0.00 | I X E | 0.01 |
| PRS : not weaned | -0.18 | -0.37 - 0.01 | Year (Intercept) | 0.16 |
| PRS : weaned | 0.03 | -0.15 - 0.21 | Residual | 0.24 |
| PDO$_{between}(\beta_B)$ | 0.09 | -0.10 - 0.29 | $R^2_{marginal}$ | 0.04 |
| PDO$_{within}(\beta_W)$ | 0.08 | -0.10 - 0.25 | $R^2_{conditional}$ | 0.48 |

Initial predictors were used in this analysis and estimates of fixed effects and variance components of random effects of models 3, 6, 9 and 12 are presented based on results from random regression analyses and LRT in Table 2.1 in main text. Within-individual centering was applied as suggested by van de Pol and Wright (2009). PRS : reproductive success the previous year

**Table A.6 Random regression analyses of the within-individual ($\beta_W$) and between-individual ($\beta_B$) components of adult female density effect on bighorn sheep parturition dates at a) low density (176 observations, 44 females over 14 years ) and b) high density (174 observations, 69 females over 11 years).**

| a) Low adult female density | | | | | | |
|---|---|---|---|---|---|---|
| Models | | Log-L | Test | d.f. | LRT | P-value |
| 1. Year + Female | | -159.76 | - | 9 | - | - |
| 2. Year + Female x Density$_{within}$ | | -159.04 | 1 vs. 2 | 11 | 1.43 | 0.49 |
| Estimates of fixed effects | Estimate | S.E. | 95% CI | Random effects | Var | |
| Intercept ($\beta_0$) | 2.74 | 0.80 | 1.19 - 4.29 | Female (intercept) | 0.03 | |
| Maternal mass in September | -0.06 | 0.05 | -0.17 - 0.03 | Year (intercept) | 0.19 | |
| PRS : not weaned | -0.29 | 0.15 | -0.58 - 0.03 | Residual | 0.29 | |
| PRS : weaned | -0.01 | 0.14 | -0.28 - 0.27 | | | |
| Density$_{between}$($\beta_B$) | 0.03 | 0.98 | -1.88 - 1.92 | | | |
| Density$_{within}$($\beta_W$ | -0.45 | 0.97 | -2.32 - 1.42 | | | |
| b) High adult female density | | | | | | |
| Models | | Log-L | Test | d.f. | LRT | P-value |
| 1. Year + Female | | -125.59 | - | 9 | - | - |
| 2. Year + Female x Density$_{within}$ | | -125.43 | 1 vs 2 | 11 | 0.32 | 0.85 |
| Estimates of fixed effects | Estimate | S.E. | 95% CI | Random effects | Var | |
| Intercept ($\beta_0$) | 2.86 | 0.16 | 2.54 - 3.18 | Female (intercept) | 0.05 | |
| Maternal mass in September | -0.12 | 0.05 | -0.23 - -0.02 | Year (intercept) | 0.09 | |
| PRS : not weaned | -0.11 | 0.13 | -0.37 - 0.15 | Residual | 0.19 | |
| PRS : weaned | 0.07 | 0.13 | -0.18 - 0.33 | | | |
| Density$_{between}$($\beta_B$) | 0.17 | 0.11 | -0.06 - 0.40 | | | |
| Density$_{within}$($\beta_W$ | 0.02 | 0.10 | -0.23 - 0.28 | | | |

Subsets are divided by the median of observed densities between 1992 and 2017; values were standardized prior to analyses. Estimates of fixed effects and variance components of random effects of models 1 are presented. PRS : reproductive success the previous year. Analyses were done on initial predictors (not detrended).

**Table A.7 Linear mixed-effects model to assess if slopes of within-individual ($\beta_W$) and between-individual environmental components ($\beta_B$) are similar or not (following van de Pol and Wright 2009, equation 3).**

| Estimates of fixed effects : Model 2 | Estimate | 95% CI |
|---|---|---|
| Intercept (no lamb) ($\beta_0$) | 2.80 | 2.62 - 3.00 |
| Maternal mass | -0.09 | -0.16 - -0.02 |
| PRS : not weaned | -0.18 | -0.36 - 0.01 |
| PRS : weaned | 0.05 | -0.13 - 0.21 |
| Precipitation$_{within}$($\beta_W$) | 0.28 | 0.15 - 0.40 |
| Precipitation$_{between}$ - Precipitation$_{within}$($\beta_B - \beta_W$) | 0.15 | -0.02 - 0.32 |
| Estimates of fixed effects : Model 5 | | |
| Intercept (no lamb) ($\beta_0$) | 2.87 | 2.67 - 3.07 |
| Maternal mass | -0.10 | -0.17 - -0.03 |
| PRS : not weaned | -0.21 | -0.39 - -0.01 |
| PRS : weaned | 0.01 | -0.17 - 0.19 |
| Temperature$_{within}$($\beta_W$) | -0.17 | -0.32 - -0.01 |
| Temperature$_{between}$ - Temperature$_{within}$($\beta_B - \beta_W$) | -0.18 | -0.34 - -0.01 |
| Estimates of fixed effects : Model 8 | | |
| Intercept (no lamb) ($\beta_0$) | 2.87 | 2.66 - 3.09 |
| Maternal mass | -0.09 | -0.17 - -0.03 |
| PRS : not weaned | -0.20 | -0.39 - -0.01 |
| PRS : weaned | 0.02 | -0.16 - 0.20 |
| Density$_{within}$($\beta_W$) | 0.04 | -0.18 - 0.26 |
| Density$_{between}$ - Density$_{within}$($\beta_B - \beta_W$) | 0.17 | -0.01 - 0.36 |
| Estimates of fixed effects : Model 11 | | |
| Intercept (no lamb) ($\beta_0$) | 2.85 | 2.62 - 3.07 |
| Maternal mass | -0.09 | -0.16 - -0.02 |
| PRS : not weaned | -0.19 | -0.37 - 0.01 |
| PRS : weaned | 0.04 | -0.14 - 0.22 |
| PDO$_{within}$($\beta_W$) | 0.09 | -0.08 - 0.26 |
| PDO$_{between}$ - PDO$_{within}$($\beta_B - \beta_W$) | 0.01 | -0.14 - 0.16 |

The random effect structure was identical to models 2, 5, 8 and 11 Table 2.1 (main text). PRS : reproductive success the previous year

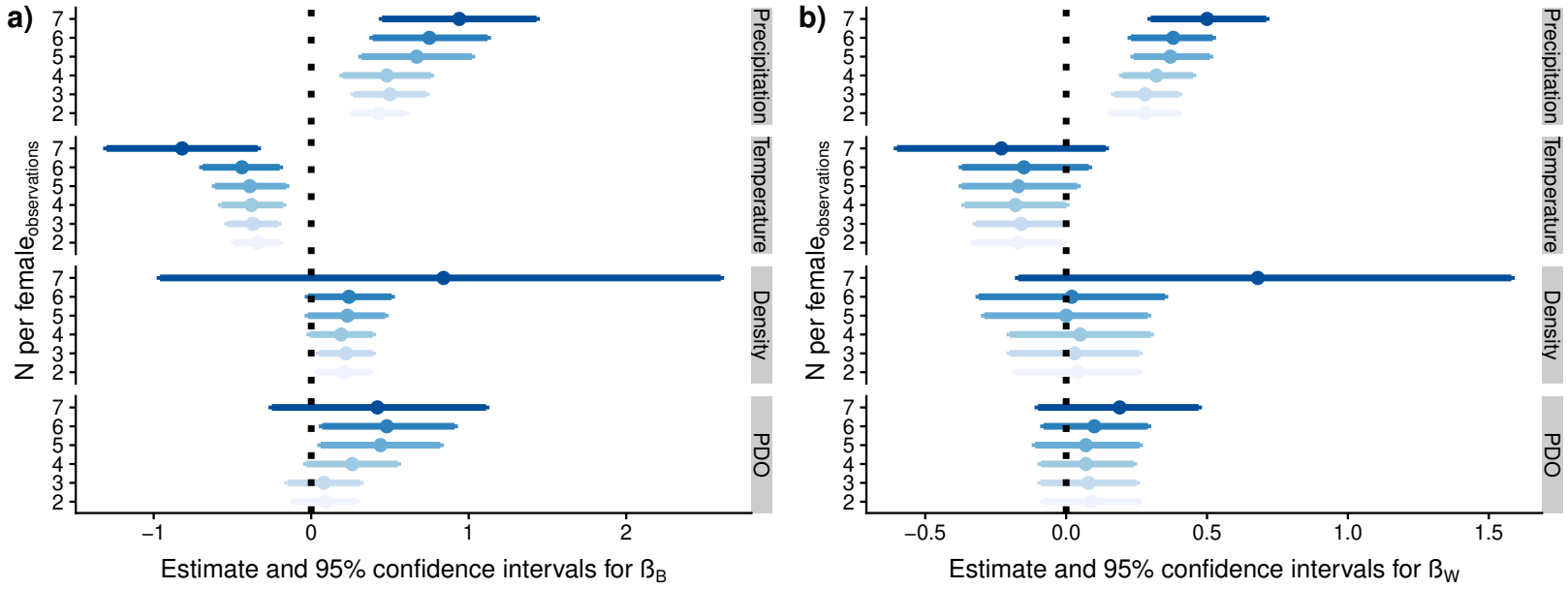


**Figure A.1 The effect of the number of measurements per female on estimates of plasticity in parturition dates in response to environmental determinants.**

We tested datasets in which number of measurements increased from 2 to 7 (indicated on the y-axis and increasingly darker colors in both panels). Between-individual components ($\beta_B$) for density, summer PDO, precipitation and temperature are illustrated on the x-axis in a) with their 95% confidence intervals for each dataset. Within-individual components ($\beta_W$) are illustrated on the x-axis in b). Mean-centering was applied as suggested by van de Pol and Wright (2009)

## A.1 References

Grosbois V, Gimenez O, Gaillard JM, et al (2008) Assessing the impact of climate variation on survival in vertebrate populations. Biol Rev 83 :357–399. doi: 10.1111/j.1469-185X.2008.00047.x

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ANNEXE B

# ANNEXE CHAPITRE 3

**Causes and short-term consequences of variation in milk composition in wild sheep**

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## B.1 Supporting methods including laboratory analyses of milk macronutrients

### B.1.1 Laboratory analyses

Milk fatty acid, protein, glucose and lactose concentrations were quantified using colorimetry, in which concentration is exhibited by a color change of the sample solution. Light absorbance of the sample can then be measured at a specific wavelength using a spectrophotometer and provide a value which is proportional to the concentration of the milk component being measured. All 96-well, flat-bottomed, polystyrene microplates (Corning, Medium binding) were from VWR (Bridgeport, NJ, USA). Plates were shaken on a MicroPlate Shaker (VWR) and absorbance was measured with a Multiskan GO microplate spectrophotometer (ThermoFisher Scientific Inc., Waltham, MA, USA). Sample fatty acid, protein, glucose and lactose concentrations were calculated and expressed as a function of milk volumes ($mg{\cdot}mL^{-1}$) and mineral concentrations are given in parts per million (weight or volume ; ppm). In all analyses, we repeated assays for any sample in which the coefficient of variation was 20% or more either between triplicates, duplicates or technical duplicates to ensure reliability of microplate lectures.

**Fatty acid analyses**

We quantified milk fatty acids using a modified colorimetric method of Atwood and Hartmann (1992) and Stern and Shapiro (1953). We gently mixed and heated milk samples to 40°C during 20 min in a hybridization oven (Boekel Scientific, USA). Six hundred $\mu$L of 100% ethanol

were added in 1.5 mL microcentrifuge tubes. To ensure repeatability between samples, technical duplicate samples (2.5 $\mu$L) and triolein standards in a 2 :1 chloroform-methanol solution (Sigma-Aldrich, St Louis, MO 63178, USA, T-7140 ; 2.5 $\mu$L) were pipetted into ethanol and vortexed. One hundred $\mu$L of hydroxylamine hydrochloride (NH2OH·HCL 2 M) and 100 $\mu$L of sodium hydroxide (NaOH 3.5 M) were added into each tube. Samples were incubated for 20 min at room temperature. One hundred $\mu$L of hydrochloric acid (HCL 4M) and 100 $\mu$L of a ferric chloride-trichloroacetic acid solution (10 mL FeCl3 0.37 M- HCl 0.l M, 7.5 g TCA) were added for color development. We vortexed tubes between each step and transferred 250 $\mu$L of each tube in duplicate on a 96-well microplate. Plates were read at 540 nm. Concentrations of fatty acids were determined by reference to a standard curve ; a linear relationship between absorbance and fatty acid concentration was observed within the range of 1.25–200 mg·mL$^{-1}$ of triolein standard with a coefficient of determination > 0.95%.

**Protein analyses**

We quantified protein concentration using Bradford (1976) and Pierce bicinchoninic acid (BCA) methods (Smith et al., 1985). Both methods require that all samples be thawed at room temperature and diluted in distilled water (1/1000 for BCA and 1/2000 for Bradford). For the Bradford method, we pipetted 150 $\mu$L of diluted samples, protein standard solutions (Bovine serum albumin ; 1.0 mg·mL$^{-1}$, Sigma-Aldrich, USA, P-0914) and distilled water in triplicate on 96-well microplates and added 150 $\mu$L of tempered Bradford solution (Bradford 1x Dye Reagent, Bio-Rad, 500-0205) to each well. Plates were shaken at 300 rpm for 30 s and incubated for 5 min at room temperature. Plates were read at 595 nm. For the BCA method, we pipetted 150 $\mu$L of sample, protein standard solutions (Bovine serum albumin ; 1.0 mg·mL$^{-1}$, Sigma-Aldrich, USA, P-0914, 150 $\mu$l) and distilled water in triplicate on 96-well microplates. BCA solution was prepared by mixing 50 mL of BCA reagent A (500 mL, ThermoFisher Scientific, USA, PI-23223) and 1 mL of BCA reagent B (25 mL, ThermoFisher Scientific, USA, PI-23224). We added 150 $\mu$L of tempered BCA solution to each well. Plates were shaken at 300 rpm for 30 s and incubated for 2 h at 37 °C in a hybridization oven (Boekel Scientific, USA). Plates were read at 562 nm. Concentrations of proteins were determined by reference to a standard curve ; a linear relationship between absorbance and protein concentration was observed within the range of 3.91–250 $\mu$g mL$^{-1}$ of protein standard with a coefficient of determination of > 0.95%.

**Sugar analyses**

Phenol in the sulphuric acid can be used for the quantitative colorimetric microdetermination of sugars (Dubois et al., 1956). We quantified milk lactose and glucose concentrations using a method modified from (Masuko et al., 2005). This method is perfectly suited for small sample volumes. Samples were diluted 1/400 in distilled water then pipetted in triplicates on a 96-well polymerase chain reaction (PCR) plates with 50 $\mu$L of standards. Sulphuric acid (150 $\mu$L H2SO4) was added to each well, rapidly followed by 30 $\mu$L of 5% phenol in water (C6H6O, Sigma-Aldrich, USA ; P4557-100ML). The mixture was heated for 5 min at 90 °C in a PCR, cooled at room temperature and transferred in a 96-well, flat-bottomed microplate. Concentrations of glucose and lactose were determined by reference to a standard curve of range 4.69–300 $\mu$g mL$^{-1}$ (Sigma-Aldrich, USA ; G6918-100ML and PHR1025-1G) with a coefficient of determination of > 0.95%.

### B.1.2 Prior definitions

As the joint modeling approach used is Bayesian, priors need to be defined for each set of estimated parameters. Note that the model does not have any intercept because all the response variables were standardized prior to performing the analysis, this is why in equation 1, the mean of the Gaussian distribution is defined as 0.

Latent variables were used to study the structure of the milk composition data presented here. To estimate both the number of latent variables, their structure and that of their associated parameters, we followed the procedure described by Bhattacharya and Dunson (2011) who proposed to use a sparse infinite factor priors. For each factor structuring a particular set of latent variables (mother's identity), the priors used were defined as follow

$$\lambda_{kj} \sim N(0, \phi_{kj}^{-1}\tau_k^{-1}) \tag{B.1}$$

$$\text{where } \phi_{kj}^{-1} \sim \Gamma(2,1) \tag{B.2}$$

$$\text{and } \tau_k = \prod_{l=1}^{k} \sim \Gamma(3,1) \tag{B.3}$$

In these prior definition, $\lambda$ refers to the parameters of the latent variables, where $\tau_k^{-1}$ is a global

shrinkage parameter for the $k^{th}$ columns and the $\phi_{kj}^{-1}$ are the local shrinkage parameters for the elements in the $k^{th}$ columns (see fig.2 in the main text). This is a multiplicative gamma process shrinkage prior on the space of covariance matrices that allows introduction of infinitely many factors, with the loadings increasingly shrunk towards zero as the column index increases. For the purpose of inference or prediction based on the covariance matrix, identifiability of the loadings is not necessary.

This prior allows for the number of latent variables to be uncertain and handles the sparse structure of the data efficiently. These priors have been shown to be efficient in both defining an adequate number of latent variables and ensuring that these latent variables are efficient in capturing the structure in the data. Note that the parameters of the gamma distribution are, in order, the shape and the scale of the distribution.

## B.2 Supporting results and analyses including full parameter estimates and their uncertainties

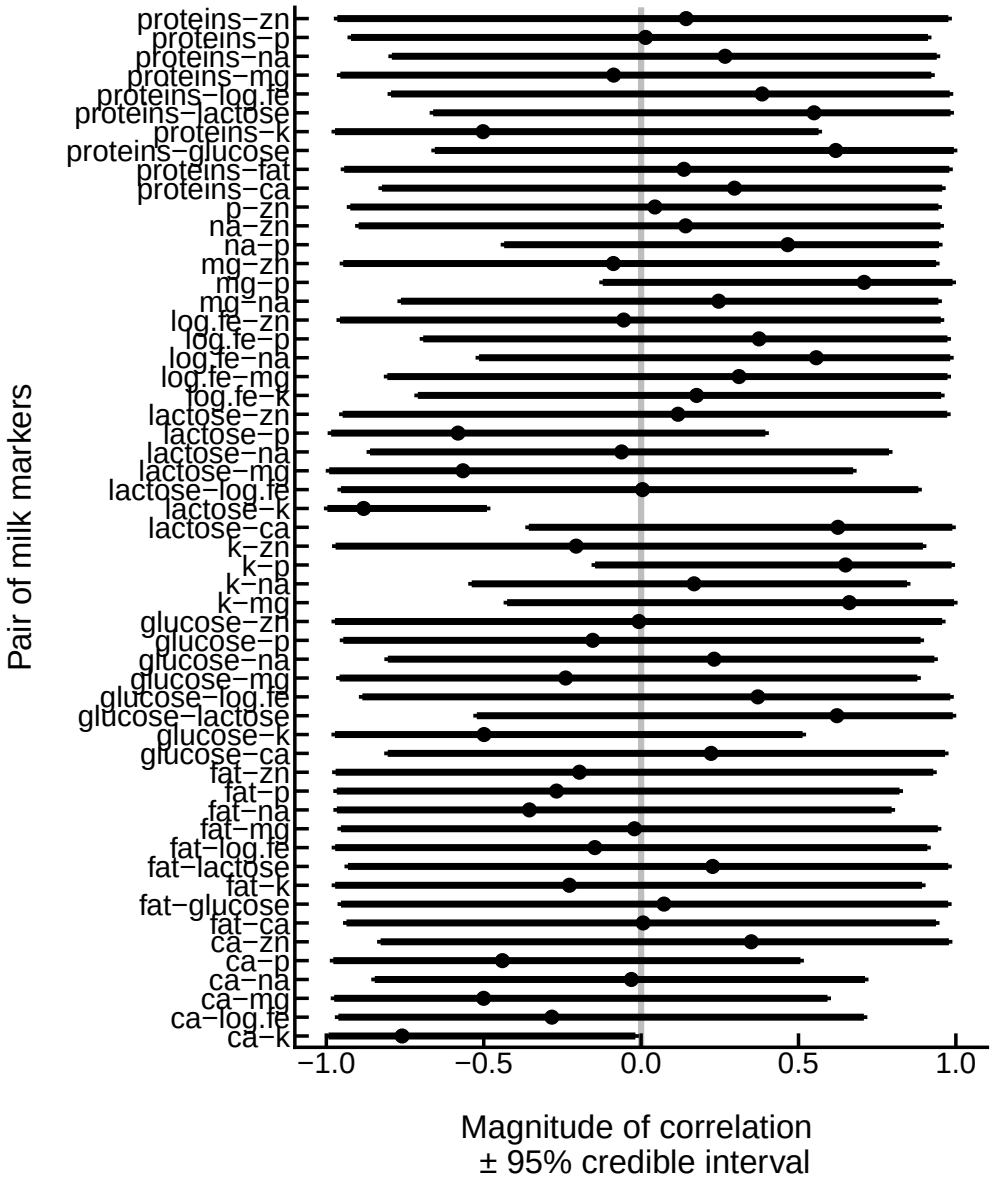


**Figure B.1 Figure showing correlation estimates and their 95% credible intervals between bighorn sheep milk components, Ram Mountain, Alberta, Canada, 2011-2016.**

Correlations were extracted at the individual level after a joint model was performed on all milk components simultaneously, with sampling year as a covariate and mother's identity and sample as random variables. Two pairs of markers had correlation estimate around which 95% credible intervals did not overlap zero. Components : Calcium (Ca), Iron (log.fe), Potassium (K), Magnesium (Mg), Sodium (Na), Phosphorus (P), Zinc (Zn)

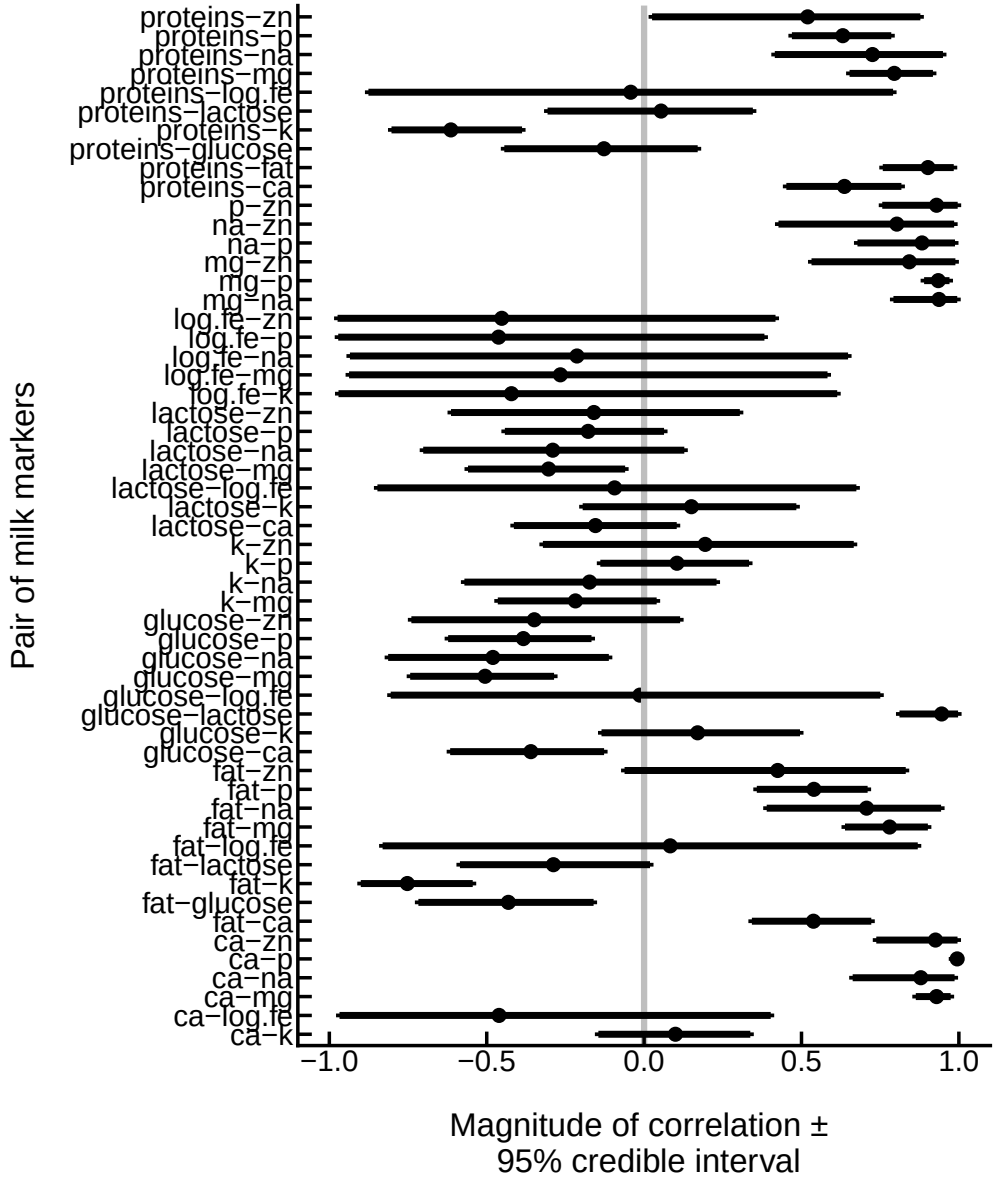


**Figure B.2 Figure showing correlation estimates and their 95% credible intervals between bighorn sheep milk components, Ram Mountain, Alberta, Canada, 2011-2016.**

Correlations were extracted at the residual (within-individual) level after a joint model was performed on all milk components simultaneously, with sampling year as a covariate and mother's identity and sample as random variables. Components : Calcium (Ca), Iron (log.fe), Potassium (K), Magnesium (Mg), Sodium (Na), Phosphorus (P), Zinc (Zn)

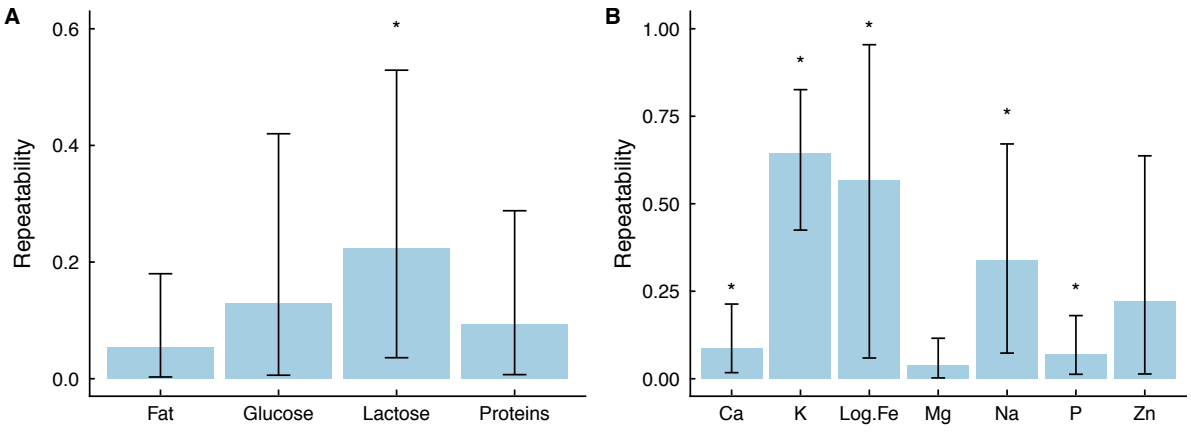


**Figure B.3 Individual repeatability of bighorn sheep milk A) macronutrient and B) mineral concentrations at Ram Mountain, Alberta, Canada, 2011-2016.**

Repeatability estimates are calculated by Bayesian joint models using the 'HMSC' package; they include individual identity and sample as random effects and sampling year as a fixed effect. Lower and upper 95% credible intervals are shown. Symbol * indicates that repeatability estimates is different from zero based on the posterior distributions (Fig. B.4). Components : Calcium (Ca), Iron (log.fe), Potassium (K), Magnesium (Mg), Sodium (Na), Phosphorus (P), Zinc (Zn)

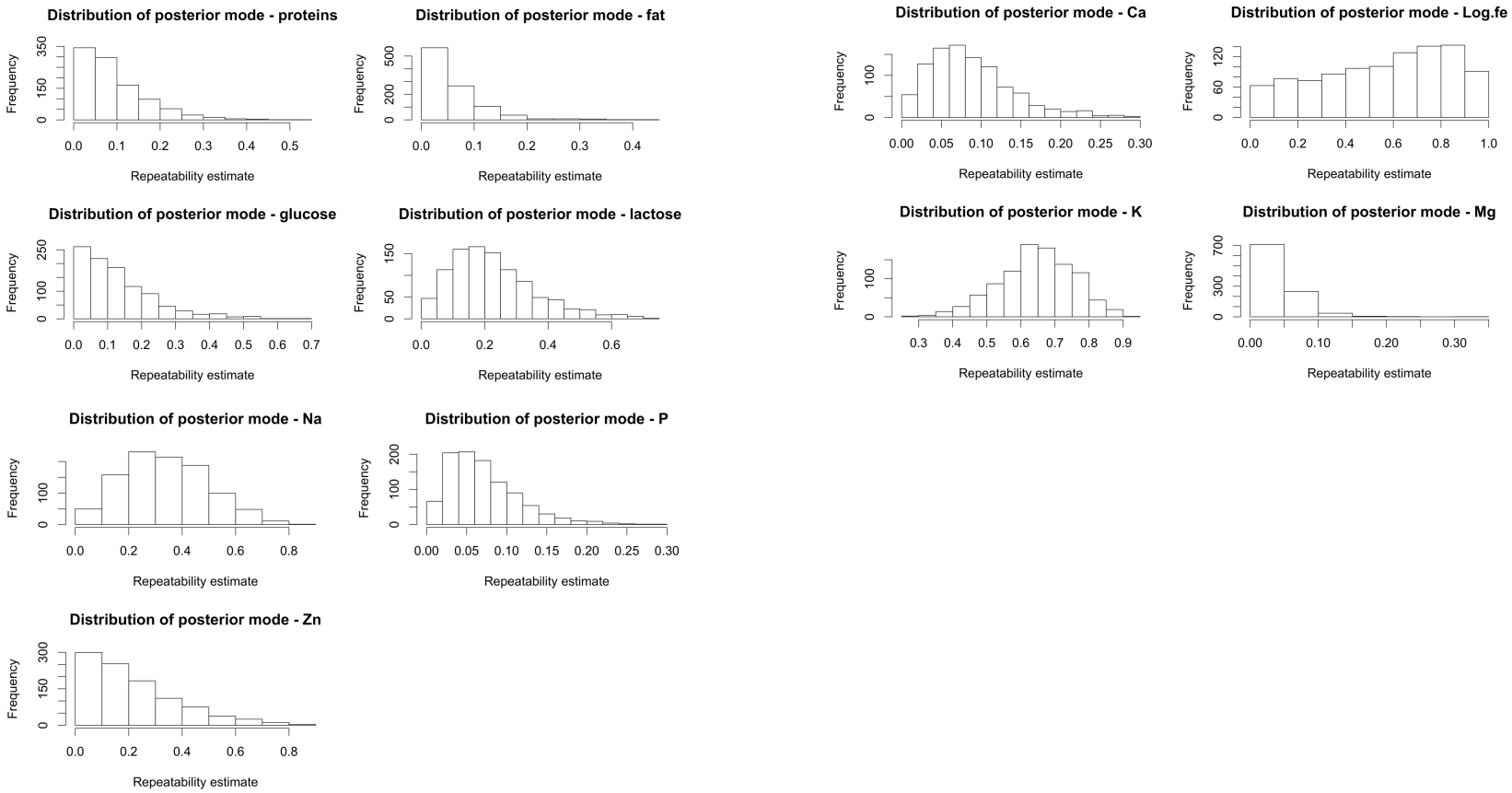




**Figure B.4 Posterior distributions of the repeatability estimates of bighorn sheep milk components at Ram Mountain, Alberta, Canada, 2011-2016.**

Repeatability estimates are calculated by Bayesian joint models using the 'HMSC' package. Components : Calcium (Ca), Iron (log.fe), Potassium (K), Magnesium (Mg), Sodium (Na), Phosphorus (P), Zinc (Zn)

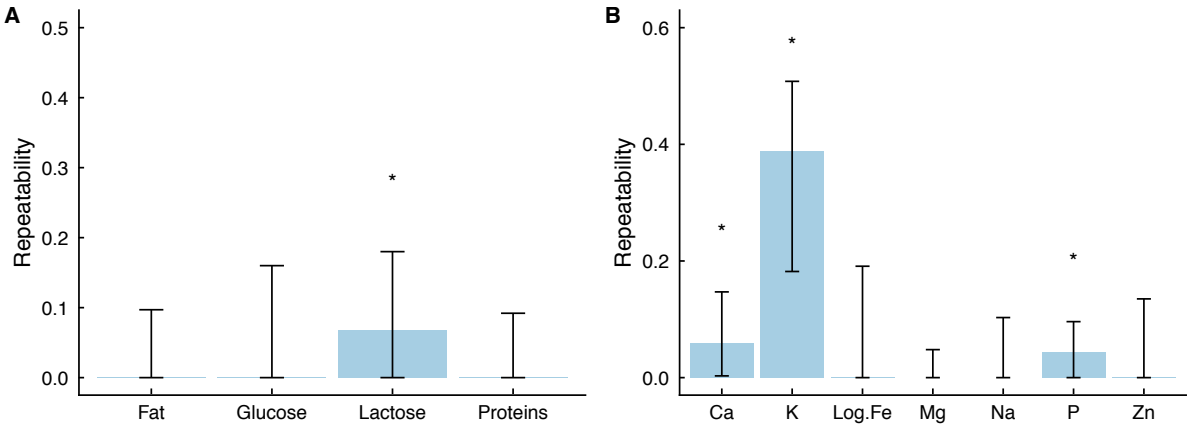


**Figure B.5 Individual repeatability of bighorn sheep milk A) macronutrient and B) mineral concentrations at Ram Mountain, Alberta, Canada, 2011-2016.**

Repeatability estimates are calculated by multivariate linear mixed-effects models using the 'MCMCglmm' package; they include individual identity and sample as random effects and sampling year as a fixed effect. Lower and upper 95% credible intervals are shown. Symbol * indicates that repeatability estimates is different from zero based on the posterior distributions (Fig. B.6). Components : Calcium (Ca), Iron (log.fe), Potassium (K), Magnesium (Mg), Sodium (Na), Phosphorus (P), Zinc (Zn)

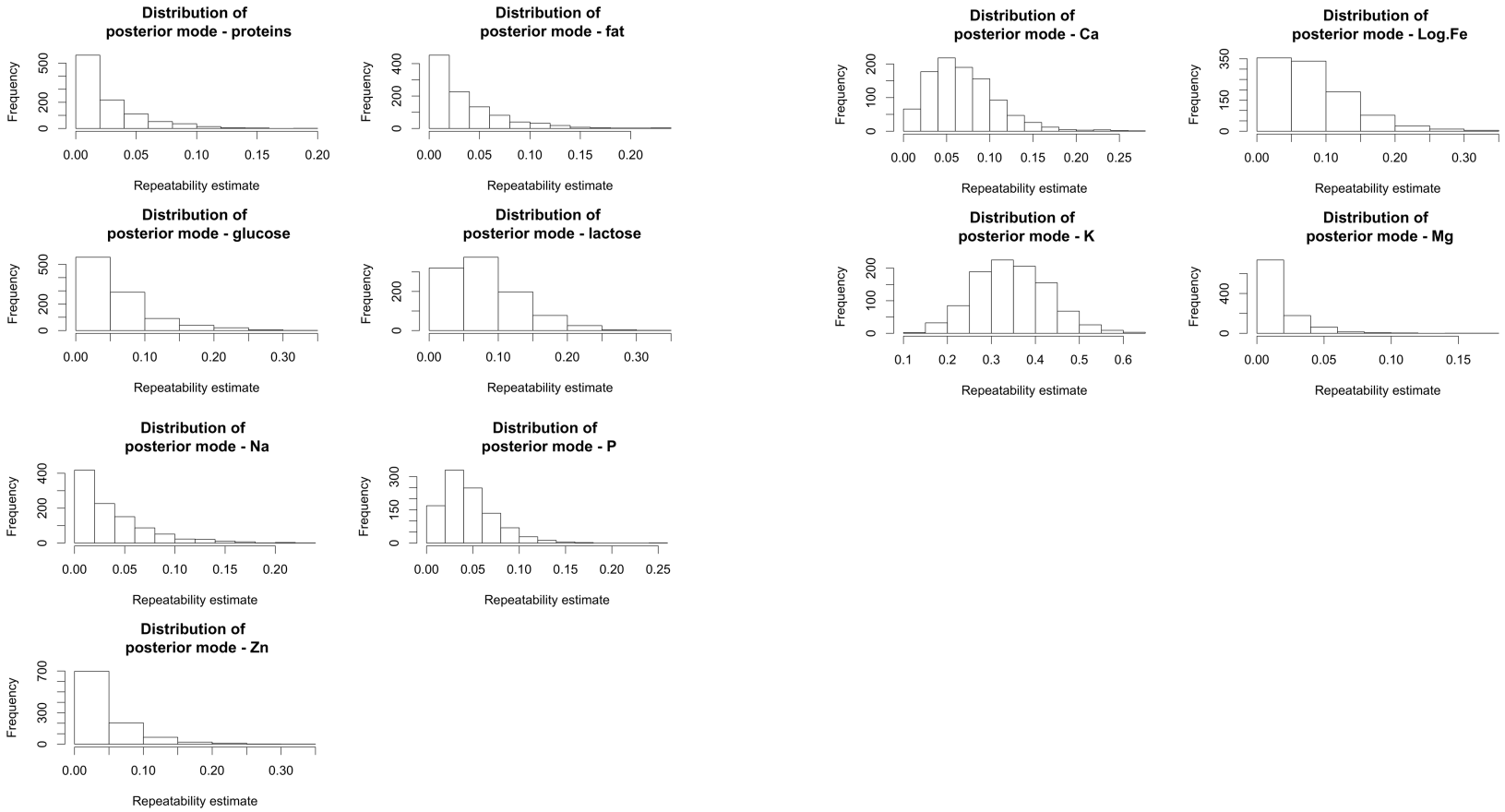


**Figure B.6 Posterior distributions of the repeatability estimates of bighorn sheep milk components at Ram Mountain, Alberta, Canada, 2011-2016.**

Repeatability estimates are calculated by multivariate linear mixed-effects models using the 'MCMCglmm' package. Components : Calcium (Ca), Iron (log.fe), Potassium (K), Magnesium (Mg), Sodium (Na), Phosphorus (P), Zinc (Zn)

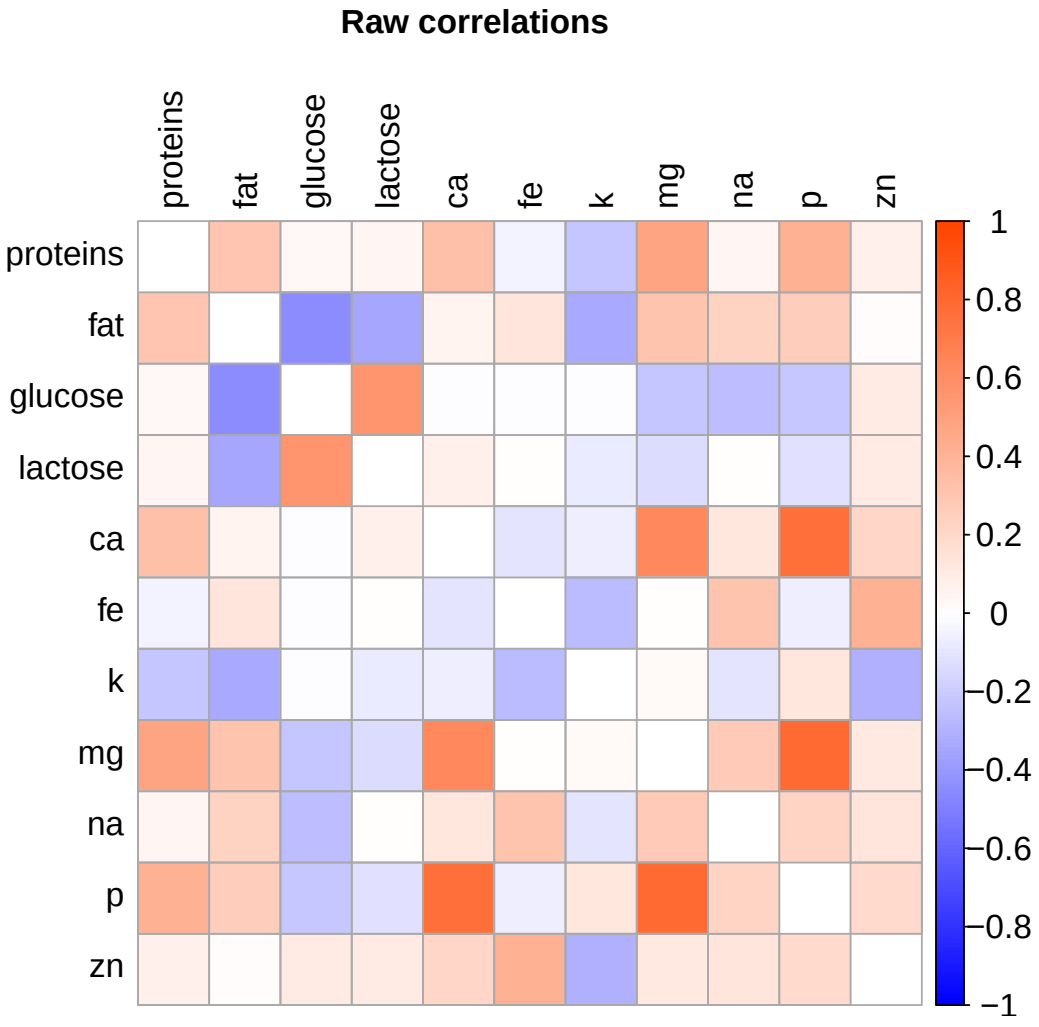


**Figure B.7 Matrix plot shows correlations between pairs of bighorn sheep milk components (raw values), Ram Mountain, Alberta, Canada, 2011 – 2016.**

Blue represents negative correlations while orange represents positive correlations between pairs of milk components. Correlations are bounded between -1 and 1 and $|r| \leq 0.4$ appear in white. Matrix plot was drawn using the 'corrplot' function from the 'corrplot' R package

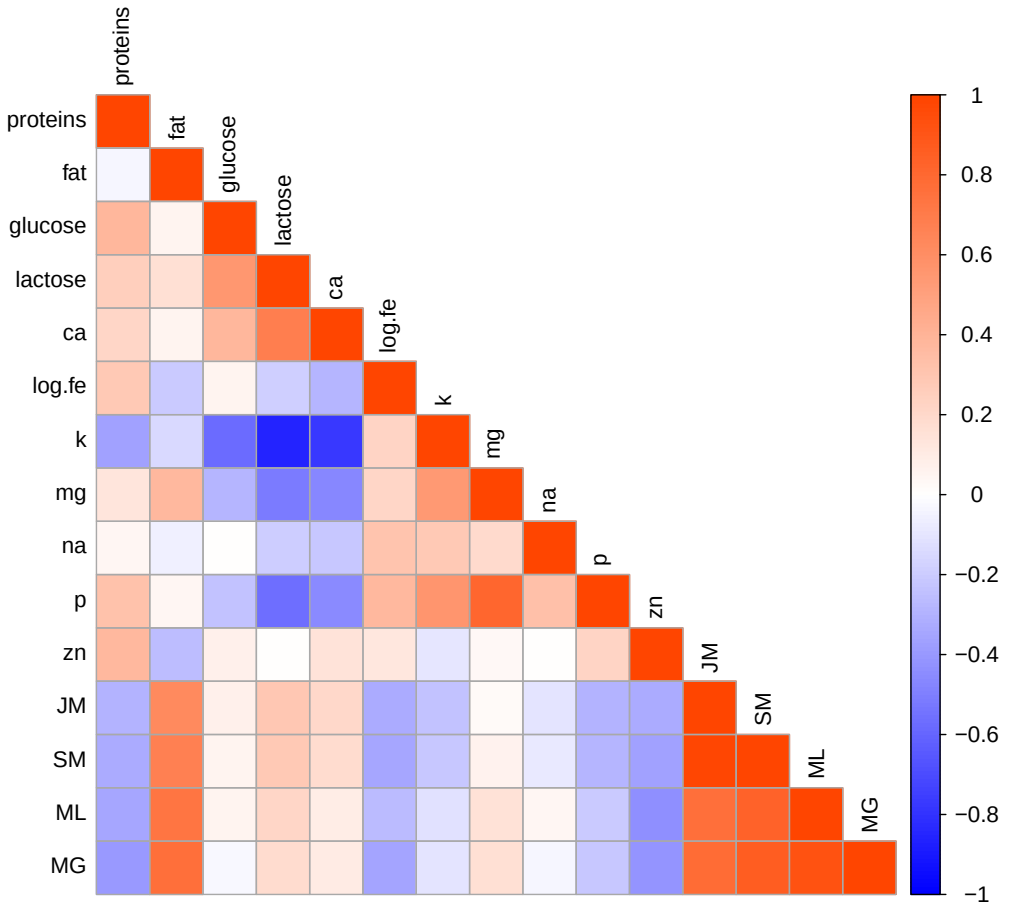


**Figure B.8 Matrix plot shows correlations between pairs of milk components among bighorn sheep mothers, Ram Mountain, Alberta, Canada, 2011 – 2016.**

Standardized June and September maternal mass, summer mass gain, i.e. the difference between September and June mass, and winter mass loss, i.e. the difference between June mass and previous September mass, were included as response variables with milk markers in the joint model. Blue represents negative correlations and red represents positive correlations between pairs of markers. Correlations are bounded between -1 and 1. 95% credible intervals around correlation estimates are partly reported in Supplementary figure B.9 for JM : June maternal mass; SM : September maternal mass; ML : Winter mass loss and MG : Summer mass gain. Components : Calcium (Ca), Iron (log.fe), Potassium (K), Magnesium (Mg), Sodium (Na), Phosphorus (P), Zinc (Zn)

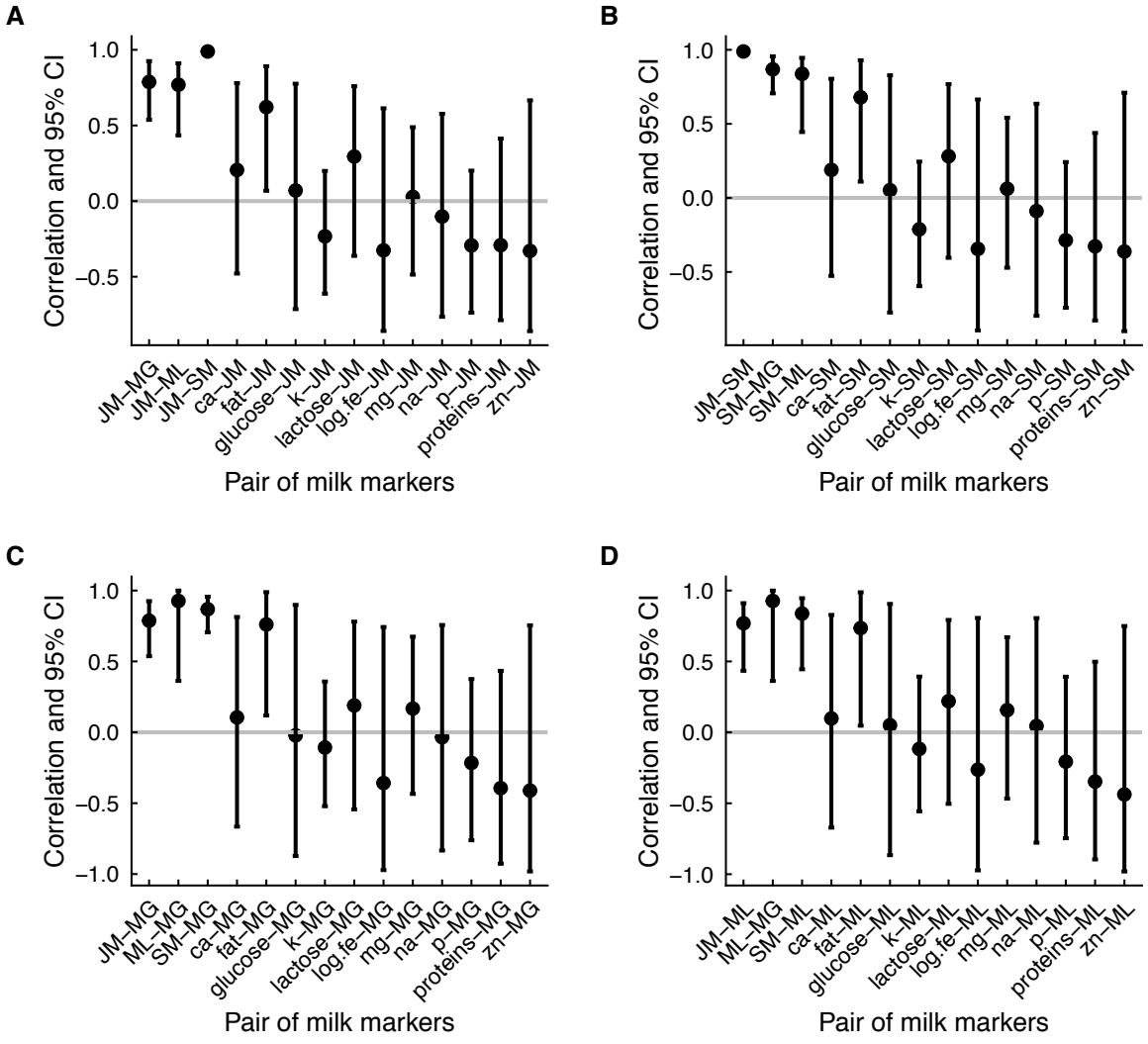


**Figure B.9 Figure showing magnitude of correlations between pairs of response variables included in the joint model reported in Supplementary figure S8.**

Correlation estimates are represented with their 95% credible intervals for all milk variables and either A) standardized June maternal mass, B) standardized September maternal mass, C) summer mass gain or D) winter mass loss. Components : Calcium (Ca), Iron (log.fe), Potassium (K), Magnesium (Mg), Sodium (Na), Phosphorus (P), Zinc (Zn). JM : June maternal mass ; SM : September maternal mass ; ML : Winter mass loss ; MG : Summer mass gain

**Table B.1 Parameter estimates and 95% credible intervals for each combination of covariates and response variables included in a joint model of bighorn sheep milk, Ram Mountain, Alberta, Canada, 2011– 2016.**

| Fixed effect | average | Lower CI | Upper CI | Fixed effect | average | Lower CI | Upper CI |
|---|---|---|---|---|---|---|---|
| Intercept | | | | Effect of lamb age | | | |
| Log.fe | –0.07 | –0.20 | 0.06 | Log.fe | –0.05 | –0.15 | 0.04 |
| K | 0.21 | 0.06 | 0.35 | K | –0.10 | –0.16 | –0.03 |
| Na | –0.23 | –0.27 | –0.18 | Na | 0.07 | 0.04 | 0.11 |
| Year 2012 | | | | Effect of June maternal mass | | | |
| Log.fe | 0.17 | 0.02 | 0.32 | Log.fe | –0.01 | –0.54 | 0.55 |
| K | –0.03 | –0.12 | 0.07 | K | –0.06 | –0.60 | 0.51 |
| Na | 0.25 | 0.20 | 0.30 | Na | –0.03 | –0.58 | 0.53 |
| Year 2013 | | | | Effect of September maternal mass | | | |
| Log.fe | –0.07 | –0.22 | 0.10 | Log.fe | –0.07 | –0.69 | 0.56 |
| K | 0.08 | –0.03 | 0.19 | K | 0.02 | –0.67 | 0.64 |
| Na | 0.15 | 0.10 | 0.21 | Na | 0.01 | –0.63 | 0.66 |
| Year 2014 | | | | Effect of previous winter mass loss | | | |
| Log.fe | –0.20 | –0.32 | –0.09 | Log.fe | 0.06 | –0.14 | 0.23 |
| K | 0.11 | 0.03 | 0.19 | K | 0.02 | –0.11 | 0.16 |
| Na | 0.11 | 0.07 | 0.15 | Na | 0.00 | –0.06 | 0.06 |
| Year 2015 | | | | Effect of summer mass gain | | | |
| Log.fe | –0.06 | –0.21 | 0.08 | Log.fe | –0.11 | –0.48 | 0.26 |
| K | 0.06 | –0.05 | 0.17 | K | 0.09 | –0.27 | 0.44 |
| Na | 0.18 | 0.13 | 0.23 | Na | –0.00 | –0.33 | 0.34 |
| Year 2016 | | | | | | | |
| Log.fe | 0.40 | 0.16 | 0.64 | | | | |
| K | –0.22 | –0.40 | –0.06 | | | | |
| Na | 0.25 | 0.18 | 0.33 | | | | |

The model contained random effects of individual identity and samples and included sampling year (a 6-level factor), lamb age, standardized June maternal mass, September maternal mass, summer or winter mass changes as fixed effects. A subset of markers was chosen based on their individual variation as response variables : Iron (log.fe), Potassium (K), Sodium (Na)

**Table B.2 Parameter estimates and 95% credible intervals for each combination of covariates and response variables included in a joint model of bighorn sheep milk, Ram Mountain, Alberta, Canada, 2011– 2016.**

| Fixed effect | average | Lower CI | Upper CI | Fixed effect | average | Lower CI | Upper CI |
|---|---|---|---|---|---|---|---|
| Intercept | | | | Effect of lamb age | | | |
| Proteins | –0.13 | –0.25 | –0.01 | Proteins | 0.41 | 0.32 | 0.51 |
| Fat | 0.08 | –0.04 | 0.19 | Fat | 0.40 | 0.30 | 0.49 |
| Glucose | –0.01 | –0.13 | 0.10 | Glucose | –0.17 | –0.27 | –0.08 |
| Lactose | –0.04 | –0.16 | 0.08 | Lactose | –0.09 | –0.19 | 0.01 |
| Ca | 0.04 | –0.05 | 0.13 | Ca | 0.28 | 0.20 | 0.35 |
| Log.fe | –0.07 | –0.18 | 0.04 | Log.fe | –0.05 | –0.14 | 0.05 |
| K | 0.19 | 0.03 | 0.33 | K | –0.10 | –0.16 | –0.04 |
| Mg | –0.07 | –0.17 | 0.03 | Mg | 0.64 | 0.58 | 0.70 |
| Na | –0.23 | –0.28 | –0.19 | Na | 0.07 | 0.04 | 0.11 |
| P | 0.07 | –0.04 | 0.18 | P | 0.46 | 0.38 | 0.53 |
| Zn | 0.04 | –0.05 | 0.14 | Zn | 0.12 | 0.03 | 0.21 |
| Year 2012 | | | | Effect of June maternal mass | | | |
| Proteins | –0.16 | –0.29 | –0.03 | Proteins | –0.07 | –0.64 | 0.45 |
| Fat | –0.01 | –0.14 | 0.12 | Fat | 0.08 | –0.54 | 0.67 |
| Glucose | 0.02 | –0.12 | 0.15 | Glucose | –0.04 | –0.60 | 0.51 |
| Lactose | 0.33 | 0.19 | 0.47 | Lactose | 0.07 | –0.49 | 0.64 |
| Ca | 0.04 | –0.06 | 0.14 | Ca | –0.01 | –0.63 | 0.50 |
| Log.fe | 0.14 | –0.01 | 0.27 | Log.fe | –0.01 | –0.56 | 0.52 |
| K | –0.04 | –0.13 | 0.06 | K | –0.07 | –0.61 | 0.45 |
| Mg | 0.05 | –0.04 | 0.14 | Mg | 0.01 | –0.64 | 0.55 |
| Na | 0.25 | 0.20 | 0.30 | Na | –0.03 | –0.56 | 0.49 |
| P | –0.03 | –0.13 | 0.07 | P | –0.06 | –0.65 | 0.47 |
| Zn | 0.02 | –0.11 | 0.16 | Zn | –0.04 | –0.65 | 0.52 |
| Year 2013 | | | | Effect of September maternal mass | | | |
| Proteins | –0.06 | –0.20 | 0.07 | Proteins | –0.04 | –0.67 | 0.63 |
| Fat | –0.02 | –0.16 | 0.13 | Fat | 0.04 | –0.67 | 0.73 |
| Glucose | 0.08 | –0.07 | 0.23 | Glucose | 0.02 | –0.63 | 0.66 |
| Lactose | 0.04 | –0.11 | 0.18 | Lactose | 0.07 | –0.58 | 0.73 |
| Ca | –0.18 | –0.30 | –0.06 | Ca | 0.03 | –0.58 | 0.73 |
| Log.fe | –0.09 | –0.24 | 0.07 | Log.fe | –0.05 | –0.68 | 0.60 |
| K | 0.06 | –0.05 | 0.17 | K | 0.01 | –0.60 | 0.65 |
| Mg | –0.10 | –0.20 | 0.00 | Mg | –0.01 | –0.65 | 0.70 |
| Na | 0.16 | 0.10 | 0.21 | Na | –0.00 | –0.60 | 0.60 |
| P | –0.20 | –0.32 | –0.07 | P | –0.02 | –0.65 | 0.66 |
| Zn | –0.05 | –0.20 | 0.10 | Zn | –0.02 | –0.67 | 0.71 |

## Table B.2 continued

| Fixed effect | Average | Lower CI | Upper CI | Fixed effect | Average | Lower CI | Upper CI |
|---|---|---|---|---|---|---|---|
| Year 2014 | | | | Effect of previous winter mass loss | | | |
| Proteins | 0.02 | –0.09 | 0.13 | Proteins | 0.01 | –0.15 | 0.17 |
| Fat | 0.07 | –0.05 | 0.18 | Fat | –0.02 | –0.19 | 0.15 |
| Glucose | –0.21 | –0.34 | –0.10 | Glucose | –0.04 | –0.21 | 0.12 |
| Lactose | –0.05 | –0.17 | 0.06 | Lactose | –0.16 | –0.32 | 0.01 |
| Ca | 0.06 | –0.03 | 0.15 | Ca | –0.01 | –0.13 | 0.11 |
| Log.fe | –0.21 | –0.32 | –0.10 | Log.fe | 0.07 | –0.11 | 0.24 |
| K | 0.10 | 0.02 | 0.19 | K | 0.01 | –0.11 | 0.13 |
| Mg | –0.02 | –0.09 | 0.06 | Mg | 0.05 | –0.07 | 0.17 |
| Na | 0.11 | 0.07 | 0.15 | Na | 0.01 | –0.05 | 0.07 |
| P | 0.00 | –0.09 | 0.09 | P | 0.03 | –0.09 | 0.15 |
| Zn | –0.22 | –0.32 | –0.11 | Zn | 0.02 | –0.13 | 0.18 |
| Year 2015 | | | | Effect of summer mass gain | | | |
| Proteins | –0.10 | –0.24 | 0.04 | Proteins | 0.03 | –0.33 | 0.39 |
| Fat | 0.48 | 0.33 | 0.62 | Fat | –0.03 | –0.42 | 0.37 |
| Glucose | –0.37 | –0.51 | –0.22 | Glucose | 0.03 | –0.36 | 0.40 |
| Lactose | –0.14 | –0.28 | 0.02 | Lactose | –0.03 | –0.40 | 0.32 |
| Ca | –0.20 | –0.31 | –0.09 | Ca | 0.04 | –0.32 | 0.38 |
| Log.fe | –0.09 | –0.23 | 0.06 | Log.fe | –0.11 | –0.49 | 0.25 |
| K | 0.07 | –0.04 | 0.18 | K | 0.06 | –0.30 | 0.41 |
| Mg | –0.10 | –0.20 | 0.00 | Mg | –0.01 | –0.38 | 0.35 |
| Na | 0.18 | 0.13 | 0.23 | Na | 0.01 | –0.31 | 0.33 |
| P | –0.09 | –0.20 | 0.03 | P | 0.03 | –0.34 | 0.41 |
| Zn | –0.52 | –0.66 | –0.39 | Zn | 0.04 | –0.38 | 0.41 |
| Year 2016 | | | | | | | |
| Proteins | –0.03 | –0.23 | 0.18 | | | | |
| Fat | 0.55 | 0.33 | 0.75 | | | | |
| Glucose | –0.26 | –0.46 | –0.06 | | | | |
| Lactose | –0.06 | –0.28 | 0.15 | | | | |
| Ca | –0.07 | –0.23 | 0.09 | | | | |
| Log.fe | 0.37 | 0.15 | 0.59 | | | | |
| K | –0.21 | –0.38 | –0.05 | | | | |
| Mg | –0.10 | –0.24 | 0.04 | | | | |
| Na | 0.25 | 0.18 | 0.33 | | | | |
| P | 0.01 | –0.16 | 0.18 | | | | |
| Zn | 0.42 | 0.22 | 0.62 | | | | |

The model contained a random effect of individual identity and included sampling year (a 6-level factor), lamb age, standardized June maternal mass, September maternal mass, summer or winter mass changes as fixed effects. All components were added in this joint model as response variables : Calcium (Ca), Iron (log.fe), Potassium (K), Magnesium (Mg), Sodium (Na), Phosphorus (P), Zinc (Zn)

## B.3 References


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ANNEXE C

# ANNEXE CHAPITRE 4

**Milk composition in a wild mammal : a physiological signature of phenological changes**

En préparation pour *Proceedings of the Royal Society B : Biological Sciences*

Limoilou-Amelie Renaud, François Rousseu, F. Guillaume Blanchet, Alan A. Cohen, Marco Festa-Bianchet et Fanie Pelletier

## C.1 Additional methods and results

We used the remotely sensed data acquired by the Moderate Resolution Imaging Spectroradiometer (MODIS) on board the NASA Terra and Aqua satellites [1]. We used seven time series as proxies of plant phenology (Table C.1). We downloaded and processed data from 2000 to 2017 using the R package 'MODIStsp' [2]. For each time series, values are available every 8 days with pixels at a resolution of 500m $\times$ 500m. Values for NDVI and EVI are available every 8 days. We considered all pixels whose centroids were within an area >1600m of elevation inside the study site.

We characterized spring and autumn plant phenology using the double logistic function suggested by [3]. The function has 6 parameters each having a biological interpretation in terms of phenology or time series values. We quantified maximal dates of 'green-up' and 'green-down' using the first and the second inflexion point of the function, respectively. These dates, in Julian day, represent the maximal increase and decrease in the time series ; we refer to these as 'green-up date' or 'green-down date', with the corresponding times series' acronym (GPP, FPAR, etc, Table C.1). We quantified the plant growing season length as the number of days between green-up and green-down, hereafter referred to 'Season length'. For the snow data, dates, in Julian day, represent the maximal rate of change in the probability of snow on the ground. We refer to spring dates as 'Snowmelt date' and to autumn dates as 'Snowfall date'. 'Season length– SNOW' was the difference, in Julian days, between 'Snowfall date' and 'Snowmelt

date' ; it approximates a 'snow-free' season. For each growing season and each pixel, we fitted the double logistic curve to the data spanning from December $12^{th}$ to March $15^{th}$.

We applied constraints on parameters of the double logistic function to avoid unrealistic fits and to improve model convergence. For each time series except SNOW, we restricted the maximal value (mNDVI) between the median and the maximum time series values. For the winter value (wNDVI), we restricted the minimum value between the minimum and the median values. Since SNOW is a binary variable (presence/absence), we restricted mNDVI to be between 0.5 and 1 and wNDVI to be between 0 and 0.5. The two inflexion points ('green-up' and 'green-down') were constrained to be between April $1^{st}$ and July $1^{st}$ and August $15^{th}$ and December $1^{st}$, respectively. Slope parameters were constrained to be between 0.03 and 0.1 except for SNOW, where values were between 0.03 and 0.5. Prior to curve fitting, we removed values that were clearly aberrant. For LAI and FPAR, we removed all values > 45 and > 90, respectively. These values seemed abnormally high and some of them appeared in the wintertime. We averaged values for each time series and each cell across the study to obtain a single value per year and per time series. We were unable to obtain convergence for very few pixel/year combinations and we ignored these cases in the calculation, assuming they had a negligible effect on the averaged dates. We fitted all curves using the 'nls' function with the 'Port' algorithm in R 3.5.0.

We investigated whether variation in plant phenology could be explained using a reduced number of variables, or dimensions, by conducting a principal component analysis (PCA) on all 7 indices. In addition to using correlations among raw variables, we used factor loadings to select those that contributed the most to the first and second principal components (PC). Since PCs are orthogonal to each other, indices that respectively contribute to PC1 and PC2 are assumed to be independent from each other.

**Table C.1 Moderate Resolution Imaging Spectroradiometer (MODIS) products used as proxies of spring phenology in 2000–2017, Ram Mountain, AB, Canada.**

| Code | Variable | Description | Range of values (in Julian day) | Product | Source |
|---|---|---|---|---|---|
| NDVI | Normalized Difference Vegetation Index | Normalized transform of the near-infrared radiation (NIR) to red reflectance ratio, $\frac{\rho NIR}{\rho red}$, designed to standardize vegetation index values to between -1 and +1. It is commonly expressed as NDVI = $\frac{NIR-Red}{NIR+Red}$ | 99 - 173 | MOD13A1 MYD13A1 | [4, 5] |
| EVI | Enhanced Vegetation Index | Same than NDVI but a canopy background correction and snow correction are applied. Extends sensitivity into dense forested biomes and agricultural areas while reducing canopy background and aerosol sources of noise. | 123 - 170 | MOD13A1 MYD13A1 | [4, 5] |
| LAI | Leaf Area Index | Defined as the one-sided green leaf area per unit ground area in broadleaf canopies and as one-half the total needle surface area per unit ground area in coniferous canopies. | 117 - 177 | MOD15A2H MYD15A2H | [6, 7] |
| FPAR | Fraction of Photosynthetically Active Radiation | Defined as the fraction of incident photosynthetically active radiation, 400-700 nanometers (nm), absorbed by the green elements of a vegetation canopy. | 118 - 179 | MOD15A2H MYD15A2H | [6, 7] |
| GPP | Gross Primary Productivity | Estimates of CO2 uptake through photosynthesis from flux towers ; it is the initial daily total of photosynthesis. | 112 - 165 | MOD17A2H MYD17A2H | [8, 9] |
| PSNNET | Net Photosynthesis | Total of photosynthesis minus all autotrophic respiration over a 24-h period. Net primary production (NPP) is the annual sum of daily PSN. | 107 - 162 | MOD17A2H MYD17A2H | [8, 9] |
| SNOW | Maximum Snow Extent | Derived from the Normalized Difference Snow Index (NDSI). Snow covered land typically has a very high reflectance in visible bands and very low reflectance in the shortwave infrared ; the NDSI reveals the magnitude of this difference.<br>Maximum snow cover extent is generated by reading 8 days of 500 m resolution MOD10A1 tiles. If snow is observed in a cell on any day in the period, the cell is mapped as snow. If no snow is found, the cell is filled with the clear-view observation that occurred most often (e.g. snow free land, lake, etc.). | 92 - 147 | MOD10A2 MYD10A2 | [10, 11] |

Range of values are, in Julian day, the maximal increase in the time series, also referred to as ‘green–up’ dates in the main text

**Table C.2 Correlations among raw phenology indices. Dates in Julian day were tested**

| | NDVI | EVI | LAI | GPP | SNOW | PSNNET | FPAR |
|---|---|---|---|---|---|---|---|
| NDVI | 1 | | | | | | |
| EVI | 0.93 | 1 | | | | | |
| LAI | 0.84 | 0.83 | 1 | | | | |
| GPP | 0.85 | 0.83 | 0.95 | 1 | | | |
| SNOW | 0.81 | 0.80 | 0.69 | 0.63 | 1 | | |
| PSNNET | 0.84 | 0.81 | 0.94 | 0.99 | 0.66 | 1 | |
| FPAR | 0.87 | 0.85 | 0.96 | 0.93 | 0.73 | 0.91 | 1.00 |

**Table C.3 A principal component analysis showing factor loadings (correlations) of all phenology indices relative to first 4 principal components (PC).**

| Phenology Index | PC1 | PC2 | PC3 | PC4 |
|---|---|---|---|---|
| NDVI | –0.36 | –0.23 | 0.52 | –0.01 |
| EVI | –0.34 | –0.23 | 0.60 | –0.12 |
| LAI | –0.40 | 0.25 | –0.27 | –0.37 |
| GPP | –0.40 | 0.37 | 0.01 | 0.31 |
| SNOW | –0.35 | –0.76 | –0.49 | 0.22 |
| PSNNET | –0.40 | 0.33 | –0.08 | 0.59 |
| FPAR | –0.40 | 0.13 | –0.21 | –0.61 |
| Cumulative proportion of variance | 0.86 | 0.95 | 0.97 | 1.00 |

**Table C.4 Estimates, standard error (SE) and *P*-values associated to all analyses of neonatal survival, including temporal trends of phenology indices.**

| | | | |
|---|---|---|---|
| **Population-level analyses** | | | |
| *Temporal trends in neonatal survival and phenology indices* | | | |
| Response variable | Explicative variables | Estimates, SE | *P*-values |
| Neonatal survival (dead / dead+live, n = 254) | Year | -0.019 ± 0.007 | 0.004 |
| Date of gross-primary productivity (GPP green-up, n = 19 ) | Year | -0.990 ± 0.771 | 0.218, |
| Date of snowmelt (n = 19) | Year | -0.780 ± 0.664 | 0.257 |
| Season length (GPP green-down – GPP green-up date, n = 19) | Year | 0.914 ± 1.036 | 0.391 |
| Season length (snowfall – snowmelt dates, n = 19) | Year | 1.097 ± 0.837 | 0.209 |
| *Determinants of neonatal survival : green-up and snowmelt dates* | | | |
| Neonatal survival (dead/dead+live, n = 15) | Intercept | 1.954 ± 0.250 | <0.001 |
| | Adult female density (N) | -0.394 ± 0.271 | 0.147 |
| | Predation (high or low) | -2.268 ± 0.576 | <0.001 |
| | Snowmelt date | 0.250 ± 0.240 | 0.297 |
| | Snowmelt date x N | -0.415 ± 0.212 | 0.051 |
| *Determinants of neonatal survival : season lengths from GPP and Snow indices* | | | |
| Neonatal survival (dead/dead+live, n = 15 ) | Intercept | 2.041 ± 0.233 | <0.001 |
| | Predation (high or low) | -2.138 ± 0.441 | <0.001 |
| | GPP season length | 0.459 ± 0.222 | 0.039 |
| | Snow-free season length | -0.427 ± 0.201 | 0.034 |
| **Individual-level analysis** | | | |
| *Determinants of neonatal survival (including all female observations)* | | | |
| Neonatal survival (0 = died, 1 = survived, n = 397) | Intercept | 7.478 ± 2.594 | 0.004 |
| | Maternal mass the previous autumn | -0.138 ± 0.245 | 0.573 |
| | Parturition date | -0.037 ± 0.017 | 0.027 |
| Relative neonatal survival (Neonatal survival – population mean) | Intercept | 0.507 ± 0.354 | 0.154 |
| n = 397 | Maternal mass the previous autumn | -0.020 ± 0.027 | 0.454 |
| | Parturition date | -0.003 ± 0.002 | 0.200 |

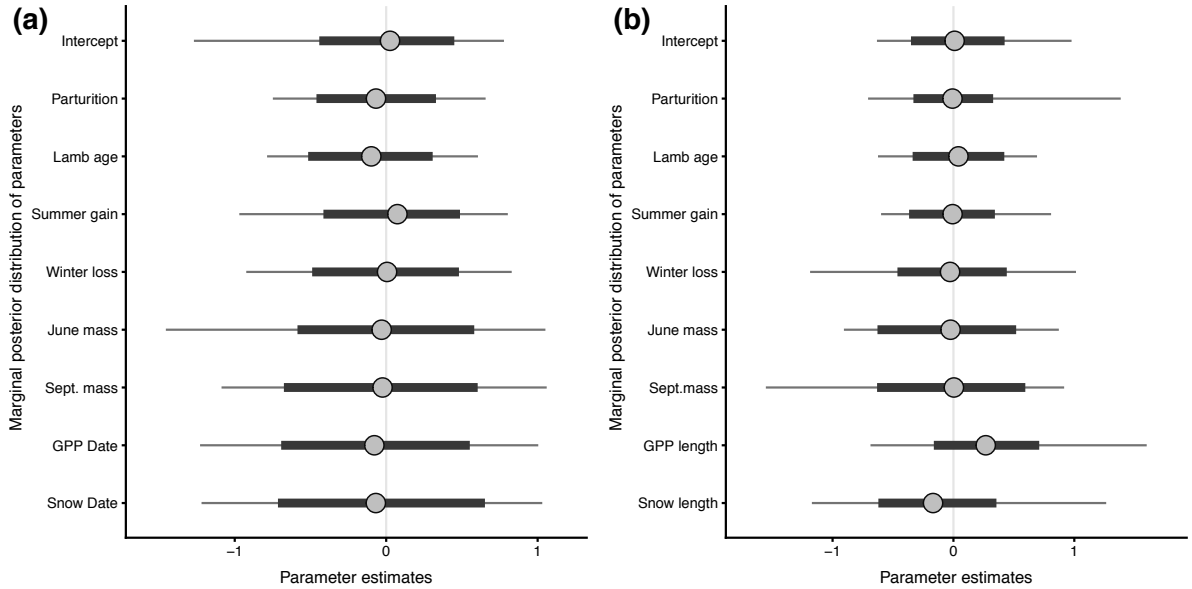


**Figure C.1 The marginal posterior distributions of regression parameters (x-axis) linking averaged milk components to spring phenology and maternal covariates (y-axis) in bighorn sheep, Ram Mountain, Alberta, Canada, 2011– 2016 (n = 220).**

*(a)* Dates of snowmelt and Green-up date (GPP) were tested as explicative variables in the multivariate model while in *(b)* "Snow-free' season length and GPP growing season length were tested in the multivariate model. Sampling year, mother's identity and a sample-level factor were all included as random effects. Parameter estimates extracted from a multivariate model of milk markers are on the x-axis ; estimates are averaged across the selected component but are presented for fatty acids, lactose and sodium concentrations in main text. The thick horizontal line represents the 95% credibility intervals and the grey dot, the mean. The vertical grey line is drawn on zero for reference.

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